CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 6 JANUARY, 1946 NUMBER 1

The Carcinogenicity of *p*-Dimethylaminoazobenzene in Diets Containing the Fatty Acids of Hydrogenated Coconut Oil or of Corn Oil*

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(Received for publication September 11, 1945)

The striking retardation in the development of hepatomas in rats fed *p*-dimethylaminoazobenzene when hydrogenated coconut oil is included in the diet has been the subject of two communications from these laboratories (6, 7). When this fat was fed at a level of 5 per cent in a synthetic diet the incidence of liver tumors was found to be only 8 per cent or less at 6 months, in contrast to a usual incidence of 60 to 80 per cent at this time when the hydrogenated coconut oil was replaced by corn oil. Although great differences exist between the chemical and physical properties of these fats (3, 7), no conclusive basis has been found for the anticarcinogenic action of hydrogenated coconut oil. Thus the presence or absence of antioxidants in these fats did not affect the tumor incidence. The effect of hydrogenated coconut oil was maintained despite the presence of amounts of ethyl linolate sufficient for the growth and health of rats on a fat-free diet. Furthermore, the formation of hepatic tumors did not depend upon the development or prevention of the syndrome characteristic of a deficiency of essential fatty acids. Only slight protection against hepatoma formation was obtained with a diet containing trilaurin at a level equivalent to the lauric acid contributed by 5 per cent of hydrogenated coconut oil. Similar results were produced with raw coconut oil. Although many small tumors in nearly normal livers were obtained with a diet free of added fat, subsequent trials have revealed that the low-fat synthetic diet generally produces a low incidence of tumors. High incidences were observed when either hydrogenated coconut oil or corn oil were fed in diets containing crude casein and rice bran extract. This is consistent with the accelerating effect that this crude diet has on hepatoma formation as compared with the synthetic diet (8).

The present study deals with the effect of the fatty acid constituents of hydrogenated coconut oil and of corn oil on the induction of liver tumors in rats fed *p*-dimethylaminoazobenzene in synthetic diets. In each case the fat was hydrolyzed and the fatty acids were fed with glycerol at levels equivalent to 5 per cent of the corresponding fat. Lauric acid, the major fatty acid constituent of coconut oil, was fed also as a sole source of dietary lipid. These groups were controlled by two others, one fed corn oil and one that received a diet free of added fat. In two smaller series diets containing either olive oil or its chief constituent, oleic acid, as the source of lipid were compared with the corn oil diet. No diets containing hydrogenated coconut oil were included in any of the three series; the tumor incidences obtained with this fat in ten previous groups have always ranged between zero and 8 per cent at 6 months (6, 7).

METHODS

As in our previous studies (6, 7, 8), young, adult, male albino Sprague-Dawley rats from 150 to 210 gm.

* This investigation was aided by a grant from the Jonathan Bowman Fund for Cancer Research. A gift of crystalline B vitamins from Dr. D. F. Robertson, of Merck and Company, is gratefully acknowledged. The hydrogenated coconut oil was obtained through the courtesy of Dr. T. M. Godfrey, of Lever Brothers Co. We are indebted to Dr. Visser van t'Hooft, of the Lucidol Corporation, for a gift of lauric acid. Thanks are due also to Mr. R. O. Johnson for aid in the preparation of the fatty acids of corn oil and hydrogenated coconut oil.

in weight were fed 0.06 per cent of *p*-dimethylaminoazobenzene for 120 days. The animals were kept in groups of 7 and 8 in screen-bottomed cages; food and water were given *ad libitum*. The dye was added to the diets by dissolving it with heat in the fat employed; for the low-fat diet an acetone solution of the dye was evaporated on the dry ration. The rations were mixed in amounts sufficient for 1 to 2 weeks and stored at 0° C. After 120 days the livers were examined by laparotomy and the animals continued on the same diet as before, but without the azo dye. At 6 months a final examination of the livers was made.

The composition of the various diets is indicated in Table I. All were supplemented with the following levels of crystalline B vitamins per kilogram: 3.0 mgm. thiamin chloride, 2.0 mgm. riboflavin, 2.5 mgm. pyridoxine hydrochloride, 7.0 mgm. calcium pantothenate, and 30.0 mgm. choline chloride. Each rat received also 1 drop of halibut liver oil monthly.

The fatty acids were prepared by hydrolysis of the original oils with alcoholic base, followed by acid decomposition of the soaps. 150 gm. of U.S.P. KOH pellets were placed in a 3 liter, round-bottom flask, 650 cc. of 95 per cent ethanol added, and the contents heated nearly to boiling on a steam bath. After 500 gm. of oil had been cautiously added to the hot solution, a condenser was attached and the mixture was refluxed for 30 minutes. The hydrolysate was cooled to about 40° C. in a stream of tap water, and 650 cc. of cold distilled water were added. With continued cooling and agitation, 275 cc. of concentrated HCl were added in small portions. The mixture was transferred to a large separatory funnel and shaken vigorously to insure complete decomposition of the soaps; the fatty acids were allowed to layer and the aqueous phase was drawn off and discarded. The acids were then washed twice with about 500 cc. of distilled water, care being taken to avoid emulsification. After drying over anhydrous Na_2SO_4 the acids were filtered through paper and 50 gm. portions placed in 125 cc. Erlenmeyer flasks. The flasks were warmed slightly to keep the acids in the liquid state, and then evacuated with an oil pump for approximately 5 to 10 minutes; after this nitrogen gas was allowed to enter, and after several exhaustions the acids were sealed under an atmosphere of nitrogen. The fatty acids were stored at 0° C., and each served for the preparation of about 1 kgm. of diet. Rancidity in the diet containing the fatty acids of corn oil was easily controlled by preparing it frequently and by storage in the cold. No attempt was made to free the fatty acids of unsaponifiable matter, and tests on the tocopherol content of the fatty acids of the corn oil indicated that about 75 per cent of this constituent remained in the acid portion.

TABLE I: COMPOSITION OF DIETS *

Constituents	Diets, gm./kgm.						
	1, 6, 8	2	3	4	5	7, 9	10
Cerelose †	790	787	786	816	840	790	795
Casein (purified ‡)	120	120	120	120	120	120	120
Salts mixture	40	40	40	40	40	40	40
Corn oil (Mazola)	50						
Fatty acids of corn oil		48					
Fatty acids of hydrogenated coconut oil			47				
Glycerol		5	7				
Lauric acid				24			
Olive oil (commercial)						50	
Oleic acid (U.S.P. XI)							45
p-Dimethylaminoazobenzene	0.6	0.6	0.6	0.6	0.6	0.6	0.6

* See text under Methods for the levels of crystalline vitamins added to these diets.
† Pure glucose monohydrate, obtained from Corn Products Refining Co.
‡ For preparation see Reference (6).

RESULTS

Most of the rats on the various diets remained in good physical condition; they gained moderately in weight, and the survival at 4 months was 100 per cent in 9 of the 10 groups (Table II). Normal incidences of tumors were observed in the control groups of the first 2 series, which were fed the diets containing corn oil (Table II, Groups 1 and 6); at 4 months the incidences were 33 and 8 per cent, and by 6 months they were 80 and 77 per cent respectively. In the third series, the control group fed corn oil had the unusually high incidence of 73 per cent at 4 months, which increased to 93 per cent at 6 months (Group 8).

When the corn oil in the diet was replaced by its constituent fatty acids (Group 2) 53 per cent of the animals developed tumors by 6 months. Since the incidence at this time on the control corn oil diet generally varies from 60 to 80 per cent, probably no great significance can be attached to the reduction apparently caused by the fatty acids of corn oil. The feeding

of the fatty acids of hydrogenated coconut oil (Group 3) lowered the incidence of hepatomas to zero, even by 6 months; this effect is comparable to our oft-repeated experience with the original glyceride (6, 7). The chief constituent of coconut oil, lauric acid, also did not allow any tumors to develop by 6 months (Group 4). A definite but less striking inhibition was obtained when a diet containing no added fat was fed; only 20 per cent of the rats had liver tumors by 6 months (Group 5). A scaly dermatitis of the paws and tail was noted in the rats of the groups whose diets lacked any source of essential fatty acid (Groups 3, 4, and 5); no dermatitis was observed in the groups receiving corn oil or its fatty acids.

The 2 series represented by Groups 6 to 10 tested the effects of olive oil and its main constituent, oleic acid, on the carcinogenicity of *p*-dimethylaminoazobenzene. When the corn oil in the control diet was replaced by olive oil a lowering in the incidence of liver tumors resulted; 33 and 53 per cent were attained by 6 months (Groups 7 and 9). In the group fed oleic acid (Group 10) the final hepatoma incidence of 87 per cent was not significantly different at 6 months from that obtained with the control diet. No dermatitis was evident in any of these groups; olive oil is known to contain a small amount of linoleic acid in ester form, and apparently the oleic acid used in these experiments also contained this factor as a contaminant.

TABLE II: THE EFFECT OF CERTAIN LIPIDS ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE

Group	Diet	Average starting weight, gm.	Average weight at 4 mos., gm.	Average food consumption gm./rat/day	Survival * at 4 mos.	Hepatomas † at 4 mos.	Hepatomas † at 6 mos.	Negative survivors at 6 mos.	Gross cirrhosis at 4 mos. none	Gross cirrhosis at 4 mos. moderate	Gross cirrhosis at 4 mos. severe	Dermatitis ‡
1	Corn oil	217	229	9.8	15/15	5/15	12/15	3	5	6	4	—
2	Fatty acids of corn oil	216	226	10.8	15/15	2/15	8/15	7	7	6	2	—
3	Fatty acids of hydrogenated coconut oil	212	229	11.0	15/15	0/15	0/15	15	15	0	0	+++
4	Lauric acid	208	218	11.0	15/15	0/15	0/15	13	15	0	0	+++
5	Low-Fat	216	232	11.5	15/15	1/15	3/15	12	14	1	0	+++
6	Corn oil	177	200	10.0	13/15	1/13	10/13	0	0	9	4	—
7	Olive oil	183	225	9.9	15/15	0/15	5/15	9	11	4	0	—
8	Corn oil	161	213	9.7	15/15	11/15	14/15	1	1	8	6	—
9	Olive oil	153	236	9.7	15/15	2/15	8/15	7	13	1	1	—
10	Oleic acid	152	207	9.6	15/15	2/15	13/15	2	13	1	1	—

* Survival = number living over number at start.
† Hepatomas = number with hepatomas over number surviving at 4 months.
‡ Dermatitis of paws and tail is indicative of a linoleic acid deficiency.

DISCUSSION

These experiments appear to support the conclusion that the fatty acids of hydrogenated coconut oil and of corn oil affect the carcinogenicity of *p*-dimethylaminoazobenzene in much the same way as do the original oils. The strong anticarcinogenic effect observed with a diet containing 2.4 per cent of lauric acid, the level of this acid equivalent to 5 per cent of hydrogenated coconut oil, suggests that it may be largely responsible for the inhibitory properties of the hydrogenated coconut oil. This is in contrast to previous experience, in which a diet containing the same amount of lauric acid in the form of trilaurin permitted a tumor incidence of 36 per cent by 6 months (7). However, coconut oil probably contains little trilaurin (3), and the lauric acid present as monolauryl- and dilauryl-glycerides may be more easily liberated in the gastrointestinal tract than that contained in trilaurin, since trilaurin melts at 46° C. whereas hydrogenated coconut oil and several of its component glycerides (3) are liquid at body temperature. The finding that stearic acid is more digestible in the form of mixed glycerides than as tristearin (5) suggests that a similar situation may hold for hydrogenated coconut oil and trilaurin. Hence the diet containing trilaurin may have resembled more closely a low-fat diet than the hydrogenated coconut oil diet; this is supported to some extent by the fact that tumors developed on both of the former diets.

Although the development of tumors was slower with olive oil and oleic acid than with corn oil, the final incidence observed with oleic acid was not significantly different from that in the control group. However, olive oil appeared to exert a small but definite inhibition.

Our present experience with the diet containing no added fat is somewhat at variance with our previous data for this diet (7). This time only 3 animals out

of 15 developed tumors by 6 months, as compared with 9 of 13 reported for the previous low-fat group. Seven of the 9 tumors in the former series were small, and were discovered only at the examination made at 6 months. A third group fed the low-fat diet developed only 23 per cent of hepatomas by 6 months (4). Hence we must conclude that our low-fat synthetic diet generally induces a low percentage of liver tumors at 6 months. Opie (9) previously reached essentially the same conclusion from experiments with a partially purified diet low in fat.

In a previous paper (7) we suggested that the observations of Frazer (1, 2) may offer an explanation for the effect of hydrogenated coconut oil. Frazer presented evidence that the partition of absorbed fat between the portal and lymphatic systems may be affected by the nature of the fat fed. Thus rats fed scarlet red dissolved in olive oil deposited dye mainly in the fat depots, especially when the action of pancreatic lipase was inhibited by feeding a detergent. When excess pancreatic lipase was fed, or when oleic acid was substituted for the olive oil, the dye was deposited chiefly in the liver. In this way a portion of the *p*-dimethylaminoazobenzene absorbed might be diverted and subjected to extrahepatic destruction. However, lauric acid and the fatty acids of hydrogenated coconut oil would be expected to divert more of the dye to the liver. The strong anticarcinogenic action of these acids does not favor this hypothesis.

SUMMARY

Ten groups of 15 rats each were fed various lipids in synthetic diets containing 0.06 per cent *p*-dimethylaminoazobenzene for 4 months, followed by dye-free diets for 2 more months. When 2.4 per cent of lauric acid or 4.7 per cent of the fatty acids of hydrogenated coconut oil were fed, no liver tumors developed by 6 months. If, however, these lipids were replaced by 5 per cent of corn oil, or by 4.8 per cent of the fatty acids of corn oil, the tumor incidences at 6 months were 80 and 53 per cent respectively. The presence of 5 per cent of olive oil or 4.5 per cent of oleic acid in the diet permitted incidences of tumors of from 33 to 53 and 87 per cent respectively at 6 months. Twenty per cent of the rats fed a diet free of added fat developed hepatomas within this time.

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Certain Effects of Dietary Fats on the Production of Liver Tumors in Rats Fed *p*-Dimethylaminoazobenzene*

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(Received for publication September 11, 1945)

Hydrogenated coconut oil and its component fatty acids appear to be the most striking examples of dietary lipid inhibitors reported for the carcinogen *p*-dimethylaminoazobenzene. When these substances were fed to rats as the sole sources of lipid in synthetic diets containing the azo dye very few liver tumors, if any, appeared by 6 months (3, 4). If, however, a fat such as corn oil was fed instead an incidence of 60 to 80 per cent at 6 months was obtained.

The effects of other fats on the carcinogenicity of *p*-dimethylaminoazobenzene has been the subject of two recent papers. György and his associates (1) have stated that Crisco and butter fat were co-carcinogenic as compared to lard when these fats were fed at high levels with diets low in protein, but no supporting data were offered. Opie (6) has compared diets containing various levels of Crisco, and has concluded that high levels of fat increase the rate of formation of liver tumors. The dye was fed continuously in these experiments and the animals that lived the longest (those on high fat) consumed more carcinogen than those that died early (those on low fat).

This report deals with the effects of the kind and the level of certain fats in the diet on the carcinogenicity of *p*-dimethylaminoazobenzene in rats fed a synthetic diet. Corn oil was compared with olive oil at a level of 5 per cent in the diet and corn oil, lard, and Crisco were tested at a level of 20 per cent. The series included also a diet free of added fat.

METHODS

Young albino Sprague-Dawley male rats, 130 to 170 gm. in weight, were fed 0.06 per cent of *p*-dimethylaminoazobenzene for 120 days. The animals were kept in groups of 7 and 8 in screen-bottomed cages; food and water were given *ad libitum*. After 120 days the livers were examined by laparotomy, and the animals (except Group 4) were continued on their previous diet without the carcinogen for another 60 days. At this time a final examination of the livers was made.

The diets consisted of purified casein (3) 120 gm.; salts mixture 40 gm.; one of the following quantities of fat: 50 gm. of either corn oil or olive oil, or 200 gm. of corn oil, rendered leaf lard, or Crisco (Primex); and cerelose (glucose monohydrate) to one kilogram. The diets were supplemented with the following levels of crystalline B vitamins per kilogram: 3.0 mgm. thiamin chloride, 2.0 mgm. riboflavin, 2.5 mgm. pyridoxine hydrochloride, 7.0 mgm. calcium pantothenate, and 30.0 mgm. choline chloride. Each rat received also 1 drop of halibut liver oil monthly. The carcinogen was dissolved with heat in the fat, or a solution in acetone was evaporated on the dry diet (Group 1). The rations were mixed in quantities sufficient for 1 to 2 weeks and stored at 0° C.

RESULTS

The data are presented in Table I. The general physical condition of the rats was good throughout the experiment until large tumors became evident. On the fat-free diet (Group 1) symptoms of fatty acid deficiency, consisting of a scaly dermatitis on the paws and tails, developed at about 3½ months; the dermatitis was absent in the other groups. The consumption of food, and hence of carcinogen, by this group was greater than for any other group, averaging 11 gm. per rat per day during the first 4 months. Only 2 rats in the low-fat group died before 4 months; 3 others developed hepatomas by 6 months, and 10 survived for this time without developing visible tumors. Gross cirrhosis at 4 months was absent or very mild. The final incidence of tumors found for this group, *viz.*, 23 per cent, was comparable to a previous experience with this diet (2).

* This investigation was aided by a grant from the Jonathan Bowman Fund for Cancer Research. We are indebted to Dr. D. F. Robertson, of Merck and Company, for a gift of crystalline B vitamins.

The inclusion of fat in the diet is shown by the other groups in the series. When 5 per cent of corn oil was added to the diet (Group 2) the incidence of tumors was 74 per cent, and increased to 94 per cent by 6 months. The figure is somewhat higher than our previous experience with this diet (3, 4, 5) would have led us to predict; in general an incidence of from 60 to 80 per cent at 6 months has been obtained in 8 previous trials. The substitution of olive oil for corn oil at a level of 5 per cent caused a substantial decrease in the incidence of hepatomas; to 13 per cent at 4 months and 53 per cent at 6 months (Group 3). Further, gross cirrhosis at 4 months was less severe on the olive oil diet, despite the fact that these 2 groups of rats consumed similar amounts of food; *i.e.*, 9.7 gm. per rat per day.

When corn oil was fed at a level of 20 per cent a noticeable increase in toxicity and carcinogenicity occurred (Group 4). The higher level of this fat caused a reduction in food consumption to 7.3 gm. per day, and this group gained the least in weight. Large tumors were palpated before 4 months, and before any were discovered in the other groups. At 4 months the livers were severely cirrhotic, and all the rats had hepatic tumors that necessitated their sacrifice at this time. The inclusion of either of two other kinds of fat in the diet at a level of 20 per cent produced results more similar to that obtained with the lower level of corn oil. The group fed Crisco (Group 5) developed 47 and 87 per cent liver tumors at 4 and 6 months respectively, and the diet containing 20 per cent of rendered leaf lard (Group 6) produced incidences of 60 and 87 per cent. The cirrhosis present in the latter 2 groups at 4 months was comparable to that found for Group 2. The intakes of food on these 2 diets were intermediate to those observed on the high and low levels of corn oil.

TABLE I: THE EFFECTS OF THE KIND AND THE LEVEL OF CERTAIN FATS ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE

Group	Diet	Average starting weight, gm.	Average weight at 4 mos., gm.	Average food consumption, gm./rat/day	Survival * at 4 mos.	Hepatomas † at 4 mos.	Hepatomas † at 6 mos.	Negative survivors at 6 mos.	Gross cirrhosis at 4 mos. none	Gross cirrhosis at 4 mos. moderate	Gross cirrhosis at 4 mos. severe
1	Low-fat	162	221	11.0	13/15	1/13	3/13	10	11	2	0
2	5% Corn oil	161	213	9.7	15/15	11/15	14/15	1	1	8	6
3	5% Olive oil	153	236	9.7	15/15	2/15	8/15	7	13	1	1
4	20% Corn oil	162	176	7.3	15/15	15/15	.....	0	0	0	15
5	20% Crisco	151	200	8.4	15/15	7/15	13/15	1	3	7	5
6	20% Lard	156	212	7.8	15/15	9/15	13/15	2	1	9	5

* Survival = Number living over number at start.
† Hepatomas = Number with hepatomas over number surviving at 4 months.

DISCUSSION

An inspection of the livers of the rats at 3 months probably would have given a more striking picture of the effect of the diet high in corn oil. The early palpation of tumors, the incidence of 100 per cent at 4 months, and the severe liver damage encountered in rats fed this diet leave no doubt as to the stimulatory action of corn oil under the conditions of these experiments. Even at a level of 5 per cent, corn oil was more stimulatory than the same level of olive oil; this comparison agrees with our previous data for these two fats (2).

The data fail to show any properties of Crisco, as compared to lard, that favor carcinogenesis, or any inhibitory effects of lard when compared to Crisco. The level of 20 per cent at which these fats were fed is close to the level of 23 per cent employed by György and his associates (1), although these workers fed the fats in a diet containing only 6 per cent of casein. Under our conditions the high levels of lard and Crisco gave results similar to those usually obtained with 5 per cent of corn oil.

Opie (6) has studied the carcinogenicity of *p*-dimethylaminoazobenzene in diets containing either no fat of 23.8, 36.5, or 68.2 per cent of Crisco. While the amount of casein in these diets differed from one another the addition of Crisco greatly increased the ultimate carcinogenic response to the azo dye. Although the rats fed the high-fat diets lived longer and consumed more carcinogen than those given the low-fat diet, the effect of added fat is nevertheless evident in the data presented by Opie. In the present experiments the dye was fed to all groups for 4 months, and the only variation in this respect was in the different amounts of food consumed by each group. The group fed the diet low in fat received the greatest quantity of dye, yet this diet was the least toxic. The addition of only 5 per cent of corn oil caused a nearly tenfold increase in tumors at the end of the period of dye feeding, while the ingestion of 20 per cent of this fat resulted in a tumor incidence of 100 per cent at this time. Furthermore, the latter group consumed the least amount of dye.

In this paper and in previous communications we

have presented data on the effects of corn oil, olive oil, coconut oil, hydrogenated coconut oil, Crisco, and lard on the carcinogenicity of *p*-dimethylaminoazobenzene in a synthetic diet. In all these experiments rats of the same strain, sex, and age were used, and the same synthetic diet was fed throughout. It is evident that the kind as well as the level of fat in the diet is important in determining the response obtained with this agent.

SUMMARY

Six groups of 15 rats each were fed 0.06 per cent *p*-dimethylaminoazobenzene for 4 months in synthetic diets containing: no fat, 5 per cent of corn oil or olive oil, or 20 per cent of corn oil, Crisco, or lard. The dye-free diets were then fed for 2 more months. A final incidence of liver tumors of 23 per cent was produced by the low-fat diet, as compared to 94 and 53 per cent respectively when the diet contained 5 per cent of corn oil or olive oil. When 20 per cent of corn oil was fed large tumors were palpated before 4 months, and the incidence of tumors at this time was 100 per cent. When 20 per cent of Crisco or lard was given, the incidence of tumors at 6 months was 87 per cent. These 2 groups were most comparable to the group fed 5 per cent of corn oil.

The data demonstrate that in general *p*-dimethylaminoazobenzene is more carcinogenic when the diet contains fat, and further show that the incidence of hepatomas is higher in rats given a diet containing 20 per cent of corn oil instead of 5 per cent. It is therefore apparent that the level of fat in the diet, as well as the type of fat, is important for the formation of hepatic tumors by *p*-dimethylaminoazobenzene.

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Desoxyribonucleic Acid in Epidermal Carcinogenesis Induced by Methylcholanthrene*

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(Received for publication July 20, 1945)

The role in cellular activities of desoxyribonucleic acid combined with proteins is well known, and the importance of this compound for normal and cancerous cells has been reviewed recently by Stowell (7). Since we have recently completed analyses of minerals in epidermal carcinogenesis (1) as a part of our investigations, it seemed desirable to determine quantitatively the desoxyribonucleic acid content of the epidermis during this transformation. The integration of the chemical, physical, and histological studies in our series has been reviewed by Cowdry (2).

EXPERIMENTAL PROCEDURE

Desoxyribonucleic acid was determined by means of the Dische diphenylamine reaction (4), as used by Seibert (6). The blue color was measured with a Coleman spectrophotometer at wave length γ 600. The standard curve was made with a very pure sample of sodium desoxyribonucleate kindly given to us by Dr. Jesse Greenstein, of the National Cancer Institute.

In extracting the nucleic acid from the epidermis we followed essentially the procedure of Johnson and Harkins (5). A sample of the epidermis was ground in a mortar with a 5 cc. portion of ice-cold distilled water until the mixture was finely divided. It was then poured into a 50 cc. centrifuge tube with the aid of two 5 cc. portions of distilled water and three 5 cc. parts of 10 per cent ice-cold sodium hydroxide. The centrifuge tube was placed in an ice bath in a refrigerator for 3 to 4 days, and stirred occasionally with a small glass rod. At the end of this time the contents of the centrifuge tube were made just acid to litmus with glacial acetic acid and centrifuged. Both filtrate and precipitate, after washing with distilled water, gave a brownish color with the Dische reagent, and the filtrate was therefore unsuitable for measuring the desoxyribonucleic (hereafter denoted by DKN) acid content. Sufficient concentrated hydrochloric acid was accordingly added to the filtrate to make it just acid to Congo red paper; a flocculent white precipitate occurred immediately, which, when filtered off, gave a strong blue Dische reaction. To the hydrochloric acid filtrate was added an equal volume of 95 per cent alcohol, but no precipitation took place. Then a portion of this alcohol mixture was neutralized and heated on a steam bath to drive off the alcohol. The Dische reaction was negative on this mixture as well as on the neutralized and concentrated hydrochloric acid solution, both results showing that all the DKN had been precipitated.

The final procedure was as follows: After precipitation of proteins with acetic acid near pH 7.0 in the centrifuge tube, the tissue residue and proteins were centrifuged and the supernatant liquid was filtered into a 150 cc. beaker. Tissue residue and proteins were washed several times with a few cc. of distilled water, centrifuged, and the supernatant fluid added to the filter paper, which was finally washed with a few cc. of distilled water. The nucleic acid was then precipitated from the well-buffered solution in the beaker by the addition of concentrated hydrochloric acid until just acid to Congo red paper, but before this was done, 5 or 6 drops of a 10 per cent solution of aluminum sulphate were added to ensure complete coagulation of the acid. The DKN was next transferred to a 50 cc. centrifuge tube, centrifuged, and then allowed to drain. To the acid in the centrifuge tube was added 12 cc. of distilled water and 24 cc. of Dische reagent (ample for the tissue samples employed). Upon stirring the acid went into solution, after which it was transferred to an 8×1 inch Pyrex digestion tube. The latter was then placed in a boiling water bath for 10 minutes to develop the blue color, a solution containing 3 cc. of distilled water and 6 cc. of Dische reagent, heated at the same time with the unknown, serving as a blank. After the tubes had been cooled to room temperature by immersion in beakers of tap water, the intensity of the blue color was measured. The blank, with little color if any, was used as a reference fluid, which automatically compensated for the same amount of color developing in the unknown in the Coleman spectrophotometer. Occasionally the blue solutions were slightly

* Aided by grants from The International Cancer Research Foundation, the National Cancer Institute, and an anonymous donor.

turbid, and then were cleared by sucking them through a sintered glass filter funnel before measuring the color intensity.

The DKN content was determined on the transplantable squamous cell carcinoma that we have used in our previous studies (1). When samples of the tumor were finely ground in a mortar and treated with an equal volume of 10 per cent sodium hydroxide, as was the epidermis, a very viscous and jelly-like solution resulted, in contrast to the freely flowing suspension obtained from normal and hyperplastic epidermis. However, the viscosity of the alkaline solution gradually diminished and at the end of 4 or 5 days' refrigeration it flowed freely, and could be treated exactly as was the epidermal suspension for the precipitation of the DKN.

New Buffalo and Swiss mice of both sexes, painted with 0.6 per cent methylcholanthrene solution in benzene as in our previous investigations (1), were employed. The animals were sacrificed 5 days after the last application of the carcinogen.

RESULTS

The results, expressed as milligrams of desoxyribonucleic acid per 100 mgm. of tissue, appear in Table I. The epidermis of normal untreated mice contained an average of 0.935 mgm. DKN per 100 mgm., while the benzene-treated had an average of 0.967 mgm. per 100 mgm. Therefore benzene alone had no appreciable effect upon the DKN content. The epidermis of mice killed 5 days after 1, 2, and 3 applications of the carcinogen in benzene contained averages of 0.584, 0.578, and 0.624 mgm. of DKN respectively; these figures are 37, 37, and 33 per cent less than the normal. The epidermis of mice sacrificed 5 days after 6 and 12 paintings had averages of 0.741 mgm. and 0.681 mgm. of DKN per 100 mgm. respectively, or 21 and 24 per cent less than normal. Five samples of squamous cell carcinoma contained 0.582 mgm. of DKN per 100 mgm., which was about 38 per cent less than normal. This decrease can be largely explained from the cellular morphology of the tumor (Tumor II of Cooper, Firminger, and Reller [10]), which is well differentiated with enlarged vesicular nuclei, and with a tendency to keratinize.

Since the DKN values for hyperplastic epidermis were below normal the following experiment was carried out according to the method of Dische (4), to ascertain whether the DKN was more firmly bound to the cell proteins in hyperplastic epidermis than in normal, and also to determine whether any of the DKN was precipitated with the proteins at pH 7 when the cold sodium hydroxide suspension of epidermis was neutralized with glacial acetic acid. Three samples of hyperplastic epidermis from mice that had received 3 paintings of methylcholanthrene on alternate days and were sacrificed 5 days after the last application, were treated with cold sodium hydroxide and the proteins precipitated with glacial acetic acid as described in the experimental procedure. After the

TABLE I: DESOXYRIBONUCLEIC ACID IN EPIDERMAL CARCINOGENESIS

Number of mice	Number of paintings	Time after first treatment to killing of mice, days		Desoxyribonucleic acid per 100 mgm. tissue, mgm.
NORMAL, UNTREATED MICE				
8				0.882
10				0.970
8				0.860
7				1.027
33 (total)			Average	0.935
BENZENE-TREATED MICE				
8	3	10		0.924
8	3	10		0.978
8	3	10		1.001
24 (total)			Average	0.967
METHYLCHOLANTHRENE-TREATED MICE				
3	1	5		0.545
3	1	5		0.623
6 (total)			Average	0.584
3	2	8		0.574
3	2	8		0.583
6 (total)			Average	0.578
3	3	10		0.618
3	3	10		0.639
4	3	10		0.615
10 (total)			Average	0.624
5	6	17		0.775
7	6	17		0.718
5	6	17		0.729
17 (total)			Average	0.741
4	12	31		0.680
5	12	31		0.708
5	12	31		0.769
4	12	31		0.656
18 (total)			Average	0.703
TUMORS				
7				0.605
5				0.523
5				0.567
5				0.595
5				0.621
27 (total)			Average	0.582

proteins and alkali-insoluble cell constituents had been washed several times with distilled water by centrifugation, to remove the DKN extracted by the cold sodium hydroxide, the proteins and cell constituents were treated with 10 cc. of $\frac{N}{2}$ sodium hydroxide and then heated on a steam bath for 2 hours. The volumes were kept constant at 10 cc. by the occasional addition of distilled water. At the end of 2 hours the solutions were neutralized with glacial acetic acid, allowed to stand for 1 hour, and filtered. To 5 cc. of the filtrates were added 4 volumes of 95 per cent alcohol, and the mixtures were allowed to stand overnight to precipitate completely any DKN that might be present. The minute amounts of precipitate were centrifuged and dissolved in water, and the Dische reaction was found to be negative on all 3 samples. The remainder of the filtrates were made just acid to Congo red paper in 15 cc. centrifuge tubes and the small amounts of precipitate were centrifuged and dissolved in water; the Dische reaction was negative on one sample, and only a barely visible bluish tint was observed in the other two. Cold sodium hydroxide therefore extracted practically all the DKN from the epidermis, and there was no evidence of a firmer combination of DKN with cell proteins in hyperplastic epidermis than in normal.

DISCUSSION

The chemical evidence for disturbance of nucleic acids in tumors has been given by Stowell (7), and need not be recapitulated here. Since our studies were confined to normal, hyperplastic epidermis, and a squamous cell carcinoma they are comparable to those of Stowell, who used a special microphotometer to measure the relative absorption of monochromatic light in sections of tissue stained by the Feulgen reaction for thymonucleic acid (8). He found that normal mouse epidermis contained more thymonucleic acid per unit volume than did hyperplastic skin (8), and the latter contained less of the acid as the hyperplasia increased. Moreover, Stowell and Cooper found that the thymonucleic acid content of human hyperplastic epidermis decreased by 24 per cent per area, whereas the mean amount of acid per cell increased in some preparations and decreased in others (9).

The decrease in the desoxyribonucleic acid content of hyperplastic mouse epidermis as measured microchemically is in agreement with these observations (8), except that in our studies the mean amount of DKN was increased with increasing hyperplasia. With respect to carcinoma, some of Stowell's specimens contained more, some less DKN per unit volume (7), while all our samples of a transplantable carcinoma had less of the acid than did normal or benzene-treated epidermis. As stated by Stowell and Cooper (9), the decrease in the DKN content of hyperplastic cells is probably due to the increased size of the cells, earlier established by Cowdry and Paletta (3).

SUMMARY

Hyperplastic epidermis contained less desoxyribonucleic acid than did normal or benzene-treated mice, and a transplantable squamous cell carcinoma had less of the acid than did normal epidermis, and about the same amount as the early hyperplasias.

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Observations on Experimentally Produced Sarcomas of Pigeons

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(Received for publication November 7, 1945)

McIntosh (3, 4) has reported the successful filtrate transmission of 5 out of 8 strains of induced fowl tumors developed and transplanted in his laboratory. A number of other investigators (5, 6, 7, 10, 11, 12) have failed to transmit a single one of something over 27 induced chicken tumors, though many of these growths were easily transplanted and metastasized freely. Recently Duran-Reynals, Shrigley, and de Hostos (2) have reported that they have had no success in attempts to transmit by filtrates 2 induced tumors of the pigeon. The present report deals with a similar result in this laboratory.

Induction of Tumors

In the past several years we have made many attempts to induce tumors in pigeons by carcinogenic chemicals. In 10 experiments, 40 pigeons were given repeated injections of 1,2,5,6-dibenzanthracene in lard or olive oil, distributed over 12 to 14 months, without a single resultant tumor. This failure may have been due to the fact that the Homing breed of pigeon used throughout this test was relatively resistant, an explanation suggested by later results. It is also true that the method of induction was not the best obtainable, as illustrated by the fact that only 23.8 per cent of chickens given dibenzanthracene in a similar manner yielded tumors; these arose late and few showed a high degree of malignancy. Subsequently it was found that greater success could be obtained by the use of a different solvent, a single injection of dibenzanthracene in benzol having induced progressive tumors in a matter of weeks in 90.6 per cent of the chickens of a large series (8). The results of injecting dibenzanthracene suspended in benzol into pigeons are given in the following report.

Six young American Domestic Flight and 2 Homing pigeons were given in each breast 0.1 cc. of a 3 per cent suspension of dibenzanthracene in benzol. Of the 8, only 2 of the Flight and the 2 Homing pigeons survived for 6 months. The 2 Flight pigeons both developed rapidly growing tumors between the seventh and eighth month after injection, whereas the Homing birds, kept under observation for 2 years, remained negative as had those in the original experiment. The 2 induced tumors, which are designated as No. 29 and No. 48, were both spindle cell sarcomas, resembled closely the induced chicken tumors and, like these, showed active invasion of muscle fibers. Autografts grew rapidly in both instances, and both proved transplantable into pigeons of the same strain bred in this laboratory. At the present time tumor No. 29, after 2 years, is in the 12th generation, and No. 48 is in the 14th. There has been a definite increase in malignancy, and they now show frequent metastases to the heart, lungs, liver, and kidneys. Occasionally, as with Chicken Tumor 18, metastases are noted in the muscles of the leg and wing. After the fifth generation the tumors were tested by transfer into market-bought Flight pigeons and found to grow readily.

Filtration Experiments

Attempts were next made to transmit these growths by filtrates. The same general methods were used in the preparation of extracts from the tumor tissues and in their filtration as those that had given positive results with chicken tumors. About 20 gm. of the fresh pigeon tumor was finely minced and then ground thoroughly in a mortar with sterile sand. To this mash was added 110 cc. of salt solution, and this suspension was then shaken vigorously for 20 minutes. The sand and larger particles were removed by centrifugation, and the supernatant fluid was passed through a Berkfeld V candle. Each pigeon was given 2 cc. of the filtrate in one breast and the same amount, with kieselguhr added, in the other breast. The details of the experiments are given in Table I.

Desiccation Experiments

Further attempts at transmission of the 2 pigeon tumors were made with desiccates. Actively growing, fresh tumor was finely minced and spread in a thin layer on the bottom of a sterile dish. This was put into a desiccator over sulfuric acid and the pressure reduced to 2 mm. of mercury. The desiccator was placed immediately in a freezing box and kept at a low temperature until the material was thoroughly dry. One gram of the pulverized desiccate was emulsified

in 5 cc. sterile water, and 0.5 to 1 cc. was injected into each breast of normal pigeons. In 10 experiments a total of 25 pigeons was inoculated with this material, without the production of a single tumor. The details are included in Table II.

by adsorption on aluminum hydroxide, and from tumor desiccates by repeated washings. The amount of inhibitor varies in different tumors, and we have had one instance, our Chicken Tumor 10, in which transmission by filtrates or desiccates was possible only

TABLE I: RESULTS OF FILTRATION EXPERIMENTS

Strain Nos.	Tumor gen.	% Takes in gen.	Amount filtrate injected, cc. Rt.	Lt.	Pigeons injected	Areas injected	Neg. results	Weeks observed
29	4th B	60	1 *	2	4	8	8	18
	5th A	80	1	2	4	8	8	18
48	6th A	100	1	2	4	8	8	16
	11th A	80	1	2	4	8	8	18
2 strains	4 exper.				16	32	32	

* The right breast injections were made with kieselguhr added to the filtrate.

TABLE II: RESULTS OF DESICCATION EXPERIMENTS

Strain Nos.	Tumor gen.	% Takes in gen.	Amount desiccate injected, cc. Rt.	Lt.	Pigeons injected	Areas injected	Neg. results	Weeks observed
29	5th A	80	0.5	1.0	2	4	4	25
	7th A	100	"	"	3	6	6	16
48	4th A	60	"	"	2	4	4	16
	5th A	80	"	"	2	4	4	16
	5th B	80	"	"	2	4	4	16
	5th B	80	"	"	2	4	4	16
	6th A	100	"	"	4	8	8	14
	7th A	80	"	"	3	6	6	16
	7th C	80	"	"	2	4	4	16
	8th B	100	"	"	3	6	6	16
2 strains	10 exper.				25	50	50	

TABLE III: RESULTS OF SERIAL EXTRACTIONS OF DESICCATE

Strain Nos.	Tumor gen.	% Takes in gen.	Serial extracts injected	Neg. results	Residues from extractions	Neg. results	Weeks observed
29	10th B	80	1st	1	Residue after 1st	1	16
			2nd	1	" " 2nd	1	16
			3rd	1	" " 3rd	1	16
			4th	1	" " 4th	1	16
			5th	1	" " 5th	1	16
			6th	1	" " 6th	1	16
48	11th D	100	1st	2	" " 1st	2	16
			2nd	1	" " 2nd	1	16
			3rd	1	" " 3rd	1	16
			4th	1	" " 4th	1	16
			5th	1	" " 5th	1	16
			6th	1	" " 6th	1	16
				13		13	

SERIAL EXTRACTION OF DESICCATED TUMORS

It has been demonstrated in this laboratory that extracts of chicken tumors may contain an inhibiting factor capable of limiting the activity of the transmitting agent in the production of tumors (9). This inhibiting factor may be removed from tumor filtrates after the removal of the large amounts of the inhibitor that this tumor contained (1).

In order to test the possibility that an inhibitor might be responsible for the negative results obtained in transmission, a number of experiments were carried out. Desiccates of the 2 pigeon tumors were sub-

jected to serial extractions by the method that had removed the inhibitor from chicken tumor desiccates. Each extract and a sample of the residue after each extraction was inoculated into pigeons of the susceptible strain. Again there were no positive results. Details of the experiments are given in Table III.

DISCUSSION

The failure to induce a single tumor among 40 pigeons by repeated injections of dibenzanthracene in lard may have been due to the resistance of the Homing strain used in these tests. Even the most effective method, a single injection of the carcinogenic chemical in benzol, which produced sarcomas in over 90 per cent of a large series of chickens and in both of 2 American Domestic Flight pigeons, failed to give any response in 2 Homing pigeons. Without a larger number of tests this result can only be considered as suggestive that the Homing strain is at least relatively resistant to the induction of tumors.

The failure to transmit the two induced pigeon tumors by cell-free material gives two more examples to be added to the large number of induced fowl tumors that have failed to be transmitted by filtrates.

SUMMARY

A single injection of 1,2,5,6-dibenzanthracene in benzol induced sarcomas in 2 of 8 American Domestic Flight pigeons and gave no response in 2 Homing pigeons. Repeated injections of the chemical in lard failed to induce a single tumor among 40 of the latter breed of birds. The two induced tumors in the Flight pigeons arose between the seventh and eighth month after injection, and grew progressively until they caused death. Both proved to be transplantable to pigeons of the same breed, and in later generations have increased in growth rate and have shown frequent widespread metastases.

Repeated attempts to transmit these sarcomas by filtrates and desiccates have uniformly given negative results.

ACKNOWLEDGMENT

Acknowledgment is made to Mr. Jerry Simunek for the technical work involved in this investigation as well as for breeding the strain of pigeons used.

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Effects of Ultraviolet Radiation on 3,4-Benzpyrene and Other Polycyclic Aromatic Hydrocarbons

I. Absorption Spectra and Some Chemical Properties of the Water-Soluble Products*

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(Received for publication September 4, 1945)

INTRODUCTION

Under the influence of ultraviolet radiation, especially in the presence of oxygen, the polycyclic aromatic hydrocarbons undergo various chemical changes, such as the well known polymerization of anthracene to dianthracene (34), or the production of photo-oxides (15, 16, 22). Some of the photo-oxidation products are soluble in water, and Boyland (7, 8) has investigated their effects on respiratory enzymes. Rideal and Schulman (39) have suggested that such water-soluble derivatives might play some part in the biological effects produced by the hydrocarbons, as they found that when the products of irradiation of a benzene solution of 3,4-benzpyrene were extracted with water, some constituent of the aqueous solution was readily adsorbed onto a protein monolayer, and might therefore be expected to pass into a living cell more readily than does the unadsorbed parent substance. A number of observations recorded in the literature lend support to this view. Lewis (32) found that when tissue cultures growing in a medium containing carcinogenic hydrocarbons were exposed to intense light on the microscope stage cell division was inhibited, and that when mitosis was restored again in the dark it resulted in abnormalities resembling those seen in malignant growths. Earle and Voegtlin (23), in similar experiments in which all but extreme red light was carefully excluded, found less degeneration in their experimental cultures than in control cultures treated with carcinogens in the presence of light. Creech (18, 19) has produced many abnormal mitoses in tissue cultures by adding the water-soluble choleic acid derivatives of carcinogenic hydrocarbons (24) to the culture medium. Mention must also be made of the extensive work of Mottram (37) on the photodynamic effect of carcinogenic hydrocarbons on infusoria; and of an observation by Hollaender and others (28) that yeast cells growing in a suspension of methylcholanthrene were destroyed by exposure to violet and near-ultra-violet light, although growth was stimulated by the hydrocarbon in the dark.

The present investigations were undertaken in the hope that they might throw light on some of these phenomena, and perhaps also on the mechanism by which the carcinogenic hydrocarbons produce tumors. It was soon found that the irradiation of 3,4-benzpyrene in benzene solution gave rise to a labile water-soluble compound together with more stable products both from the solute and from the benzene solvent itself (1). These substances could be recognized by means of their absorption spectra, which differ from that of any derivative yet recorded, and their aqueous solutions have been found to be carcinogenic (2). Substances with similar absorption spectra have been produced photochemically from other polycyclic aromatic hydrocarbons. Under the conditions of the experiments, the yields were too small to allow of chemical analysis, but they sufficed for examination by a technic based on absorption spectrophotometry, and some of the chemical properties have been deduced. The chemical aspects of the work are described in this and the succeeding paper (5); the biological aspects will be discussed in Part III (3).

EXPERIMENTAL

Materials.—The following hydrocarbons have been examined: naphthalene, anthracene, cholanthrene, 1,2,5,6-dibenzanthracene, phenanthrene, and 3,4-benzpyrene. The specimens were the purest obtainable, having been prepared either by Roche Products, Ltd., or in the laboratory of Professor J. W. Cook.[1] The solvents used were "spectroscopically pure" cyclohexane (33), and benzene "for molecular weight determination" (British Drug Houses).

* Because of the difficulties of international communication the authors have not read proof of this article.

[1] We are indebted to Dr. J. C. Mottram and to Dr. J. H. Schulman for gifts of pure materials.

Irradiation.—The solutions were contained in open Petri dishes, area 52 sq. cm., placed symmetrically 3 inches below a neon-sensitized mercury lamp of the type designed by Melville (36), from which 90 per cent of the energy radiated is concentrated at 2,537 A. The light absorbed by the solutions was thus virtually monochromatic; and the number of quanta incident on 1 sq. cm. of liquid surface per second, measured by Forbes' uranyl oxalate actinometer (11, 31), was about 10^{18}. The volume of solution taken was 10 ml. All the energy is absorbed in a shallow surface layer, so that a larger volume gave no advantage; smaller quantities tended to dry out and the reaction could not be controlled. A concentration of about $M/500$ of hydrocarbon was found to be convenient, though this could be varied over a fairly wide range without affecting the yields, as would be expected if the concentration was high compared with the number of quanta utilized. The irradiations usually lasted from 20 to 30 minutes, the yield not varying much between these limits.

Extraction of the water-soluble photoproducts.—At the end of the irradiation the solvent was quickly evaporated in a current of air at room temperature, and the residue was at once extracted by rubbing with a glass rod with 10 ml. of either $N/100$ HCl or $M/100$ $NaHCO_3$, and filtered through a Whatman No. 1 filter paper. The clearest filtrates were obtained from acid extracts, and this procedure was generally adopted, the acid solutions being made alkaline when required by adding excess of bicarbonate.

This method of filtration did not prevent the passage into the filtrate of minute quantities of suspended hydrocarbon. This was estimated by shaking the filtrate with a measured excess of cyclohexane, when the hydrocarbon passed into true solution in the organic layer and its concentration could be determined spectrophotometrically. In a series of experiments with extracts from 3,4-benzpyrene, the maximum concentration recorded was 2.73×10^{-6} gm. per ml. of the aqueous solution.

Absorption spectra.—For measurement of the absorption spectra of irradiated cyclohexane solutions, the volume lost by evaporation was restored by adding fresh solvent; but when the irradiated solution contained benzene, the whole solvent was evaporated off and the residue was redissolved in the original volume of cyclohexane. The aqueous extracts required no treatment after filtration.

The intensities of the absorption bands were measured with a Hilger "Spekker" spectrophotometer, and a tungsten-steel spark was used as light source. For recording the sharp bands of 3,4-benzpyrene, a medium-sized Hilger quartz spectrograph, giving their positions to ± 2 A., was available; for the much wider bands of the water-soluble derivatives, an intermediate-sized Hilger quartz spectrograph sufficed, and the positions of the maxima could be estimated to ± 10 A.

The values of optical density, $d = \log_{10}$ (incident intensity/transmitted intensity), plotted in Figs. 2, 3, 4, and 5, and quoted in Tables II and III, were calculated on the basis that the irradiation products from 10 ml. of initial solution were contained in 10 ml. of filtrate, and refer to a 1 cm. column of absorbing solution.

EFFECT OF IRRADIATION ON THE ABSORPTION SPECTRUM OF 3,4-BENZPYRENE

The absorption curve for 3,4-benzpyrene dissolved in cyclohexane drawn in Fig. 1, curve 1, is similar in intensities to, but reveals a little more detail than, that recorded by Mayneord and Roe (35) for a solution in ethanol. Under irradiation, the intensity of the whole spectrum at first diminished. The structure then became less clear (curve 2), and the absorption spread towards longer wave lengths, the solutions

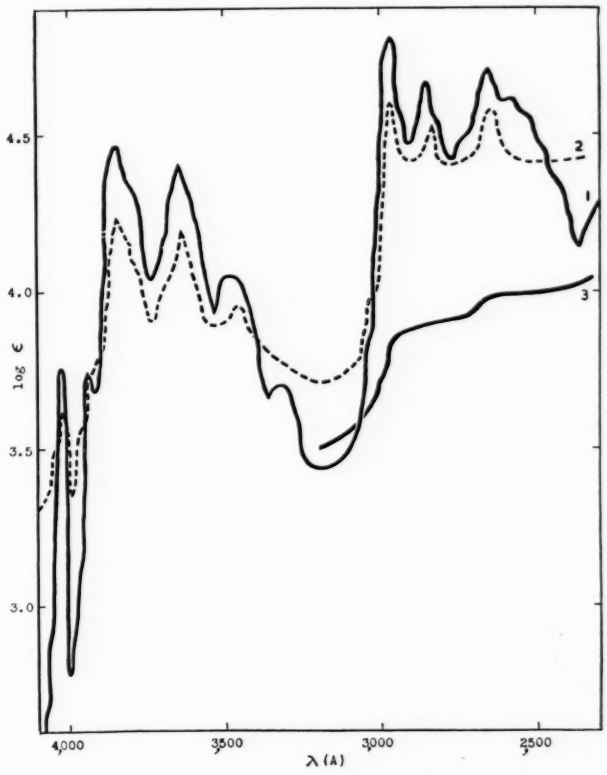

FIG. 1.—Effect of irradiation on the absorption spectrum of 3,4-benzpyrene.

Curve 1. Absorption spectrum of 3,4-benzpyrene in C_6H_{12} solution
" 2. Absorption spectrum of 3,4-benzpyrene after 1 hour's irradiation
" 3. Absorption spectrum of 3,4-benzpyrene after 7 hours' irradiation

(Irradiated solutions contained 1.45 mgm. in 100 ml.)

becoming yellow. Prolonged irradiation led to complete disappearance of the characteristics of the spectrum (curve 3). These changes are in accordance with the oxidative nature of the photoreaction. The rate of disappearance of benzpyrene depended on the concentration of the solution (Table I) as would be expected

TABLE I: PHOTODECOMPOSITION OF 3,4-BENZPYRENE IN CYCLOHEXANE SOLUTION

Concentration, per cent (w/v)	Duration of irradiation, hours	Percentage decomposed, ± 2
0.3	1.5	11
0.03	4	17
0.03	10	80
0.015	1	15
0.015	7	Absorption characteristics destroyed

if the reaction were confined to a shallow surface layer on account of the strong screening effect of the solute.

When 5 per cent of benzene was added to the solvent, only 3 per cent of the benzpyrene in a 0.3 per cent solution was decomposed in 90 minutes as compared with 11 per cent in the absence of benzene, while less color developed in the solutions. This retardation of the reaction could be attributed to screening by the benzene, which absorbs light of the same wave lengths as the solute. Subsequent investigation has shown that some of the benzene itself undergoes photochemical change (1, 4, 5).

ABSORPTION SPECTRA OF THE WATER-SOLUBLE PRODUCTS

I. INFLUENCE OF THE IRRADIATION SOLVENT

1. *Irradiation in pure cyclohexane solution.*—Acid extracts from each of the hydrocarbons after irradiation in pure cyclohexane showed little selective absorption; but when they were made slightly alkaline with bicarbonate, two types of spectrum could be recognized (Fig. 2). The spectra of the photoderivatives from naphthalene and phenanthrene contained indications of a badly defined band at about 2,700 A., with little other absorption (curve 1). The corresponding solutions from anthracene, 1,2,5,6-dibenzanthracene, and cholanthrene exhibited a sharp band at 3,600 A. (curve 2). The spectrum of the benzpyrene derivative fell between these two types, as it contained a weaker band at 3,600 A. with an incipient band at 2,700 A. (curve 3).

2. *Irradiation in solvents containing benzene.*—Quite different results were obtained when the irradiation solvent was pure benzene or a mixture of benzene and cyclohexane. Under these conditions all the acid extracts showed a well defined band at 2,760 A., and the alkaline (bicarbonate) extracts showed both this band and one at 3,600 A. (Fig. 3, curves 1 and 3).

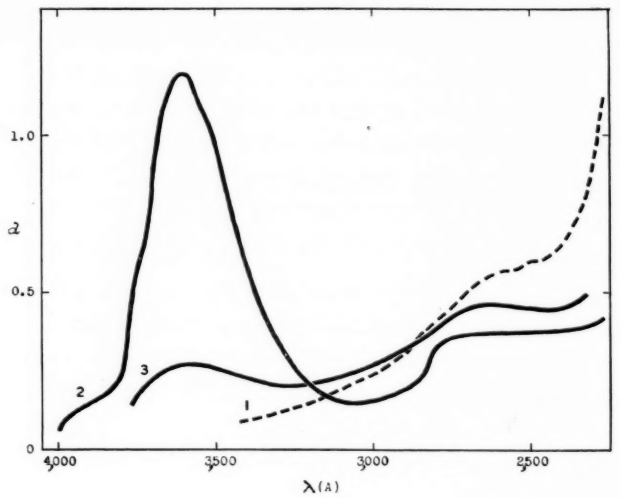

FIG. 2.—Absorption spectra of aqueous extracts (pH 8) from hydrocarbons irradiated in cyclohexane solution.

Curve 1. Naphthalene type
" 2. Anthracene type
" 3. 3,4-Benzpyrene

TABLE II: ABSORPTION BANDS OF EXTRACTS AT PH 8 FROM POLYCYCLIC AROMATIC HYDROCARBONS AFTER IRRADIATION IN CYCLOHEXANE

Parent hydrocarbon	Band at 3,600 A. λ_{max}	Band at 3,600 A. d_{max}
Naphthalene	....	...
Phenanthrene	....	...
Anthracene	3,600 A.	1.20
1,2,5,6-Dibenzanthracene	3,600	0.92
Cholanthrene	3,600	0.65
3,4-Benzpyrene	3,600	0.28

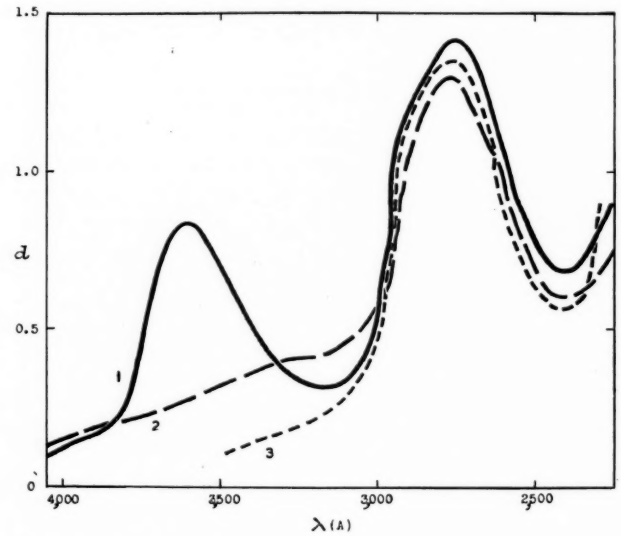

FIG. 3.—Absorption spectra of aqueous extracts from hydrocarbons irradiated in benzene solution.

Curve 1. Alkaline extract, pH 8
" 2. " " after decay
" 3. Acid extract, pH 2

The intensities of the bands at 3,600 A. were not affected by varying the concentration of the benzene from 5 per cent to 60 per cent, but with more than 60 per cent of benzene present the band became both less intense and less defined. Changes of the benzene concentration between 100 per cent and 10 per cent had little effect on the band at 2,760 A. The data set out in Table III were obtained when the concentration of benzene was 20 per cent, this being the one normally adopted. Table III shows that while the wave lengths of the two bands of the photoderivatives vary little from hydrocarbon to hydrocarbon, their relative intensities as observed at pH 8 show considerable variations.

TABLE III: ABSORPTION BANDS OF EXTRACTS FROM POLYCYCLIC AROMATIC HYDROCARBONS IRRADIATED IN A MIXTURE OF 1 PART BENZENE + 4 PARTS CYCLOHEXANE (V/V)

Parent hydrocarbon	Band at 3,600 A. Extract at pH 8		Band at 2,760 A. Extract at pH 8		Band at 2,760 A. Extract at pH 2	
	λ_{max}	d_{max}	λ_{max}	d_{max}	λ_{max}	d_{max}
Naphthalene	3,600 A.	0.68	2,760 A.	3.18	2,760	3.17
Phenanthrene	3,600	0.69	2,740	1.49	2,740	1.51
Anthracene	3,600	0.78	2,760	1.21	2,760	1.27
1,2,5,6-Dibenzanthracene	3,600	0.65	2,760	1.95	2,760	2.30
Cholanthrene	3,600	0.68	2,780	0.79	2,780	0.77
3,4-Benzpyrene	3,600	0.83	2,760	1.43	2,760	1.38

3. *Role of the benzene in the photoreactions.*—These results suggest strongly that the benzene itself entered into the reactions, since (a) the intenser band at 2,760 A. was observed only when benzene had been present; and (b) the presence of benzene was necessary in the cases of naphthalene and phenanthrene, and to a less extent in that of 3,4-benzpyrene, for the formation of a substance absorbing at 3,600 A. The function of the benzene, which further experiment showed to be complex, is discussed in Part II (5).

II. Influence of the pH of the Aqueous Solutions

The band at 3,600 A. in the spectra of the water-soluble products of irradiation of all the hydrocarbons appeared only when the solutions were neutral or, preferably, slightly alkaline. With a freshly prepared solution the band could be made to appear and disappear, without loss of intensity, by alternating the pH from 2 to 8. If, however, the solutions were made strongly alkaline (pH 14), a new band at 3,800 A. was superposed on that at 3,600 A., and the band at 2,760 A. disappeared irreversibly.

III. Stability of the Irradiation Products

1. *Bicarbonate solutions.*—When the slightly alkaline solutions were allowed to stand, the band at 3,600 A. rapidly disappeared, whereas that at 2,760 A. retained its intensity for a much longer time (Fig. 3, curves 1 and 2). The rate of decay of the 3,600 A. band depended on the temperature and on the presence of oxygen, and varied with the parent hydrocarbon. (a) *Temperature.* At 20° C., the intensity for the benzpyrene derivative fell to half-value in 4 hours, while that of the anthracene derivative decreased by only 40 per cent in the same time. At 0° C., one-third of the intensity for the benzpyrene derivative, and one-seventh of that for the anthracene derivative, had disappeared in 4 hours. (b) *Exclusion of oxygen.* When nitrogen, washed free from oxygen, was bubbled through the solutions continuously at 20° C., 22 per cent of the intensity of the band for the benzpyrene derivative, and 30 per cent of that for the anthracene derivative, disappeared in the first 4 hours. (c) *Combined effect of cooling and exclusion of oxygen.* When the bicarbonate solutions were kept in a current of nitrogen at 0° C., much greater stabilization was achieved. The band at 3,600 A., both for the benzpyrene and for the anthracene derivative, was 15 per cent *more* intense after 4 hours than it was initially at room temperature. This increase was apparently achieved at the expense of a decrease of 20 per cent in the intensity of the band at 2,760 A. (Fig. 4). After

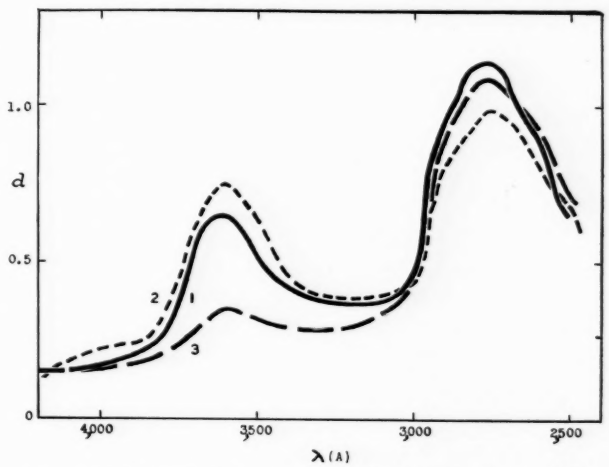

FIG. 4.—Effect of cooling to 0° C. in an atmosphere of N_2 on the absorption spectrum of a bicarbonate extract from irradiated anthracene.

Curve 1. Initial solution
" 2. Experimental solution after 4 hours
" 3. Control solution (in air at 20° C.) after 4 hours

24 hours, only 20 per cent of the intensity at 3,600 A. had disappeared, as compared with the whole band under ordinary conditions. (d) *Effect of reducing agents.* The band at 3,600 A. could not be restored by the action of any simple reducing agents on decayed solutions.

TABLE IV: EFFECT OF COOLING AND EXCLUSION OF OXYGEN ON THE STABILITY OF PHOTODERIVATIVES FROM ANTHRACENE AND FROM 3,4-BENZPYRENE AT pH 8

RATIO OF OPTICAL DENSITY TO VALUE AT ZERO TIME

Time, hrs.	Band at 3,600 A.		Band at 2,760 A.	
	$C_{14}H_{10}$	$C_{20}H_{12}$	$C_{14}H_{10}$	$C_{20}H_{12}$
4	1.15	1.15	0.90	0.90
24	0.80	0.80	0.64	0.78

2. *Acid solutions.* The acid extracts, the spectra of which contained only the band at 2,760 A., were more stable. When they were made alkaline after standing for 24 hours, the intensity of the 3,600 A. band that was developed was comparable with that for a decayed bicarbonate solution after 4 hours.

IV. PHYSICAL SEPARATION OF TWO ABSORBING IRRADIATION PRODUCTS

These changes in the absorption spectra under varying conditions indicated that the aqueous solutions contained at least two constituents, one of which absorbed at 3,600 A. and the other at 2,760 A. The presence of two such substances in the extracts from anthracene after irradiation in benzene solution was confirmed by extracting the aqueous solutions with organic solvents, after it had been found that fractional crystallization was impossible on account of the instability of the dry substances, and that chromatography was unduly complicated by the large excess of unchanged anthracene.

More concentrated aqueous solutions than had previously been used were obtained by extracting the products from 4 or 5 separate but simultaneous irradiations with the same 10 ml. of dilute HCl. The following observations were then made: (a) The solution was shaken for several minutes with an equal volume of benzene, and the absorption spectrum of the aqueous layer was recorded both before and after the shaking. Four-fifths of the intensity of the 2,760 A. band, but only one-quarter of that at 3,600 A., had been removed. (b) The acid extract was made alkaline and shaken with ether. Most of the substance absorbing at 2,760 A. passed into the ether, while that absorbing at 3,600 A. remained quantitatively in the alkaline solution. (c) Re-extraction of the organic layers with water confirmed that no chemical change had occurred. (d) The water extract of the ethereal layer from (b) was made strongly alkaline (pH 14). The band at 2,760 A. disappeared and was replaced by one with a maximum at 3,800 A. This confirmed the observation made with the original acid extracts.

REACTION OF THE PHOTODERIVATIVES WITH COMPOUNDS CONTAINING SULFHYDRYL GROUPS

When an acid extract from irradiated 3,4-benzpyrene was neutralized with a bicarbonate solution that contained cysteine (1 mgm. cysteine hydrochloride per ml., freshly dissolved), it was found that the intensity of the absorption band at 2,760 A. was considerably reduced as compared with that for a normal control solution. Other aminoacids, *e.g.,* glycyl tyrosine, gave no such effect, but a very definite suppression of the band was caused by neutralization with bicarbonate containing glutathione (0.7 mgm. per ml.), as is shown

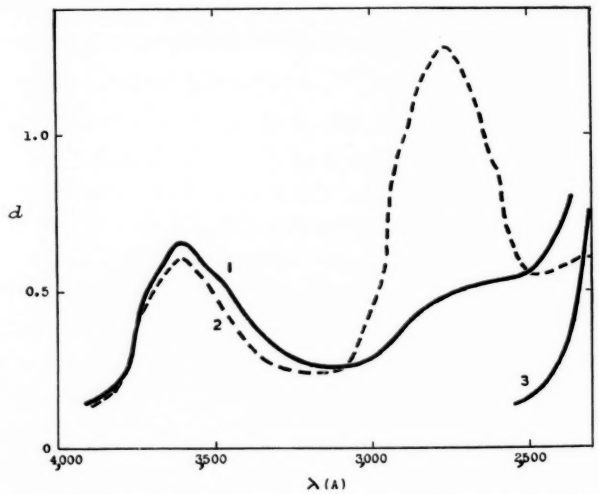

FIG. 5.—Reaction of photoderivative of 3,4-benzpyrene with glutathione.

Curve 1. Alkaline extract containing glutathione
" 2. " " control
" 3. Glutathione solution

in Fig. 5, and also with thioglycollic acid. Similar results were obtained with the anthracene photoderivative. It may be concluded, therefore, that the substance that absorbs at 2,760 A. in each case is capable of reacting rapidly at room temperatures with sulfhydryl groups. Whether this is a specific property, or indicates only that the substance can exert oxidizing powers, will be investigated in later experiments.

NATURE OF THE WATER-SOLUBLE PRODUCTS

The most striking feature of the absorption spectra of the aqueous extracts obtained after irradiation of all the six hydrocarbons that have been studied is the identity in the wave length of the 3,600 A. band produced in each case. The slight displacements of the maxima of the bands at 2,760 A., also, are only just at the limits of experimental accuracy. This almost

complete identity of position, though not of intensity (Table III), suggests strongly that the hydrocarbons all give rise to the same two water-soluble products. Such a conclusion would not necessarily be invalidated by (a) differences in the "backgrounds" of the respective spectra, (b) variation in the rates of decay of the 3,600 A. band, and (c) the differences in the relative proportions of the two substances produced, since each of these factors could be determined by the presence of other, not identical, products not specifically revealed by the absorption spectra. Until the substances have been separated and identified chemically, however, no final conclusion is justified; but it is clear that even if the various products are not identical they are very similar chemically, and the following information can be deduced about the two principal ones:

Substance A.—This could exist in two forms, the equilibrium between them depending on the pH and the temperature. In alkaline solution, at 0° C., a form absorbing at 3,600 A. predominated but at higher temperatures it passed over into one that absorbed weakly at shorter wave lengths. Both forms were unstable and readily took up oxygen when they decomposed. In acid solution, only the form absorbing at shorter wave lengths existed, and it was then more stable.

Such behavior might be expected if the substance was a weak acid, which, while undissociated in acid solution, formed ionized salts in alkaline solution. The ion would absorb at 3,600 A., and the undissociated molecule at 2,760 A. Differences between the absorption spectra of an undissociated acid and its ionized salts are not uncommon; *e.g.,* picric acid (26).

The undissociated form had equal solubilities in ether and in water, and was twice as soluble in the latter as in benzene, so that it was probably aliphatic. The long wave length of the absorption band of the ion suggests that it contained a considerable degree of unsaturation. Its production would involve extensive breakdown of the parent hydrocarbon skeleton.

Substance B.—This was more soluble in benzene than in ether, and was freely soluble in water, so that it was probably oxygenated and aromatic. It had an absorption maximum at 2,760 A. It was more stable to oxygen than was substance *A,* but it was destroyed by strong alkali, and reacted very readily with substances containing -SH groups. Its formation during the photoreactions depended on the presence of benzene, but it will be shown in Part II (5) that it was most probably not formed from the benzene alone.

SPECTROSCOPIC ATTEMPTS TO IDENTIFY SUBSTANCES *A* AND *B*

The absorption spectrum of substance *A* does not coincide with that of any likely compound recorded: that of substance *B* occurs at wave lengths that are "unspecific," but usually associated with simpler aromatic compounds.

Two types of oxidized photoproducts of polycyclic hydrocarbons seemed probable; *viz.* (a) bridged-ring compounds, and (b) hydroxyderivatives.

1. *Bridged-ring compounds.*—That any of the substances now described were of the anthracene photo-oxide type (21, 34) was excluded by the work of Cook, Martin, and Roe (15, 16), who showed that such compounds cannot be obtained from 1,2,5,6-dibenzanthracene or from 3,4-benzpyrene. Such photo-oxides are in any case insoluble in water. Water solubility is

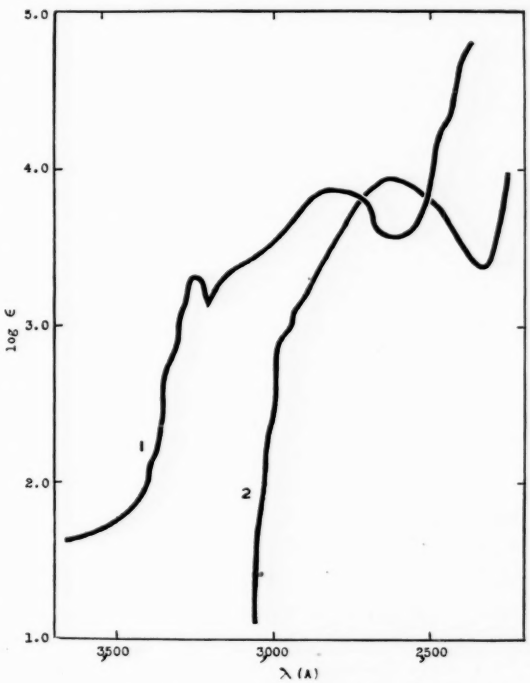

FIG. 6.—Absorption spectra of:

Curve 1. Na-endosuccinate of 1,2,5,6-dibenzanthracene
" 2. 1,2-dihydro-1,2-dihydroxy-naphthalene

obtained when the ring is bridged by an acid group, and the spectrum of the endosuccinnate of 1,2,5,6-dibenzanthracene is shown in Fig. 6, curve 1. It is typical of an anthracene molecule divided into halves by saturation of the 9,10-carbon atoms (17), and contains a band at 2,780 A., but it also has a subsidiary maximum at 3,250 A.

2. *Hydroxyderivatives.*—A number of water-soluble hydroxyderivatives of polycyclic hydrocarbons are now well known, *e.g.,* 1,2-dihydroxy-1,2-dihydroanthracene (9), dihydroxy-dibenzanthracene (10, 12, 20), and 8-hydroxy-3,4-benzpyrene (6, 13, 14); but the absorption spectra of such compounds usually resemble closely those of the parent hydrocarbons, with the finer structure obliterated (6, 10, 12, 20, 25, 27, 29, 30, 40). For comparison, the spectrum of an alkaline solution

of 1,2-dihydroxy-1,2-dihydronaphthalene (prepared by Professor Drummond) is reproduced in curve 2 of Fig. 6.

The behavior of solutions containing substances *A* and *B* must also be compared with that of the products of prolonged irradiation of polycyclic aromatic compounds that were described by Boyland. These were soluble in water and inhibited lactic dehydrogenase *in vitro* (7) and also respiration and anaerobic glycolysis of animal tissues (8), the effects increasing with the duration of the irradiation (up to 24 hours) and with pH (up to 12). They were acid substances, stable in strong alkali. This excludes the possibility that they were identical with substance *B*.

Some interest also attaches to *α*-ethyl-*β*-*sec*-butyl stilbene (21), which has the same carbon skeleton as 3,4-benzpyrene and might therefore represent an intermediate stage in the breaking down of the benzpyrene molecule. Such stilbenes, however, have absorption bands at the wrong wave lengths; *i.e.*, at 3,000 A. (38).

The identity of the substances *A* and *B* thus remains unestablished; but further attempts are being made to isolate them, and to identify them by chemical methods.

SUMMARY

1. Under the influence of monochromatic ultraviolet radiation of wave length 2,536 A. naphthalene, anthracene, phenanthrene, cholanthrene, 1,2,5,6-dibenzanthracene and 3,4-benzpyrene in solution in cyclohexane formed water-soluble products.

2. When the irradiated solutions also contained benzene, the absorption spectra of the water-soluble derivatives from these substances were sufficiently similar to suggest identity of the respective photoproducts.

3. At pH 8 aqueous solutions of the products gave rise to bands whose maxima were at 3,600 A. and 2,760 A. respectively, and the two bands were found to originate from two separate substances.

4. The substance absorbing at 3,600 A. was unstable, and readily took up atmospheric oxygen. Its behavior suggested that it might be an unsaturated acid, and it was possibly aliphatic.

5. The substance absorbing at 2,760 A. was more stable, was oxygenated, and was aromatic. It reacted readily with compounds containing -SH groups, and was destroyed by strong alkali.

6. Neither substance could be identified spectroscopically with any known derivative of 3,4-benzpyrene or of similar compounds.

ACKNOWLEDGMENTS

This work was financed by grants to one of us (C. B. A.), which we gratefully acknowledge; at first from the Medical Research Council and latterly from the British Empire Cancer Campaign. We also have pleasure in thanking Dr. H. B. Fell and Professor E. K. Rideal, F. R. S., for their continual interest and advice, and for providing facilities in their laboratories.

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Effects of Ultraviolet Radiation on 3,4-Benzpyrene and Other Polycyclic Aromatic Hydrocarbons

II. The Role of Benzene in the Photoreactions in Solutions Containing It*

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(Received for publication September 4, 1945)

INTRODUCTION

In the preceding paper (3) it was shown that when naphthalene, anthracene, 1,2,5,6,-dibenzanthracene, cholanthrene, phenanthrene, or 3,4-benzpyrene was irradiated with radiation of wave length 2,537 A. in the presence of benzene, water-soluble materials were produced that gave rise to very similar absorption spectra. The spectrum of slightly alkaline solutions contained two sharp bands with maxima at 3,600 A. and 2,760 A. respectively and these could be attributed to the presence of two distinct compounds, named for convenience substances *A* and *B*. Comparison with the results obtained when the hydrocarbons were irradiated in solution in pure cyclohexane alone established that (a) the intenser band at 2,760 A. was observed only when benzene was present during the irradiation; and (b) the substance absorbing at 3,600 A. was formed from naphthalene and phenanthrene only when benzene was present. The role of the benzene in producing these results is now discussed.

THE PHOTO-OXIDATION OF LIQUID BENZENE

Under the same conditions of irradiation, benzene, either as pure liquid or when dissolved in cyclohexane at concentrations of over 20 per cent (v/v), also yielded water-soluble products (1, 2) that were much more stable than those of the polycyclic compounds. The absorption spectra of the aqueous solutions, both acid and slightly alkaline, contained a well defined band at 2,760 A., but the spectrum between 3,000 A. and 4,000 A. exhibited strong, unselective absorption, the highest intensity in which was reached at about 3,950 A. (Fig. 1, curve 1). The wave length of this maximum depended only slightly on the pH of the extracting solution and on the concentration of benzene during the irradiation; its intensity diminished a little in the first few hours after the extraction, but then became constant. The band at 2,760 A. showed constant intensity with time, and was not affected by pH up to that of the bicarbonate solutions,. but it was destroyed irreversibly in strongly alkaline solution (pH 14).

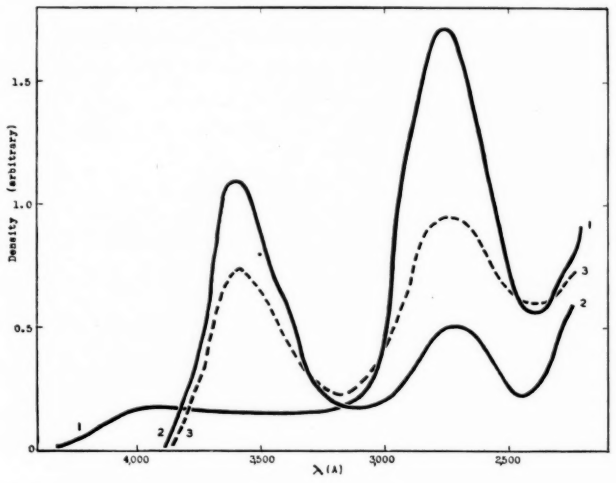

FIG. 1.—Absorption spectra of aqueous extracts from irradiated benzene.

Curve 1. 20% C_6H_6 in C_6H_{12}
" 2. 0.01% C_6H_6 in C_6H_{12}
" 3. The same after standing

(Densities for curve 1 have been divided by 5)

EXISTENCE OF SECONDARY REACTIONS

These results suggested that the band at 2,760 A. in the spectra of the photoderivatives from the polycyclic compounds was due to the benzene only; but this was partly contradicted by the observation that the intensity of the band was much higher when benzene was irradiated alone than when 3,4-benzpyrene was irradiated in it. Reaction between the benzene derivative and the benzpyrene solute was thus probable.

Direct confirmation of such a reaction was obtained in an experiment in which (a) benzpyrene was

* Because of the difficulties of international communication the authors have not read proof of this article.

irradiated in cyclohexane, and (b) pure benzene was irradiated separately. The unchanged benzene in (b) was dried off, and the residue, taken up in cyclohexane, was added to the irradiated solution from (a). After 4 hours the solvent was again evaporated at room temperature, and the residue was extracted with *N*/100 HCl. The absorption spectra of extracts from solutions (a) and (b) are drawn in curves 1 and 2 respectively of Fig. 2, and that for the final extract in curve 3. It closely resembles that for an extract from 3,4-benzpyrene after irradiation in a solution containing benzene, as shown in Fig. 3 of the preceding paper.

The probability still remained that part, at least, of the intensity of the band at 2,760 A. in the spectra of the substances derived from the polycyclic hydrocarbons was due to a derivative of benzene; but this conclusion

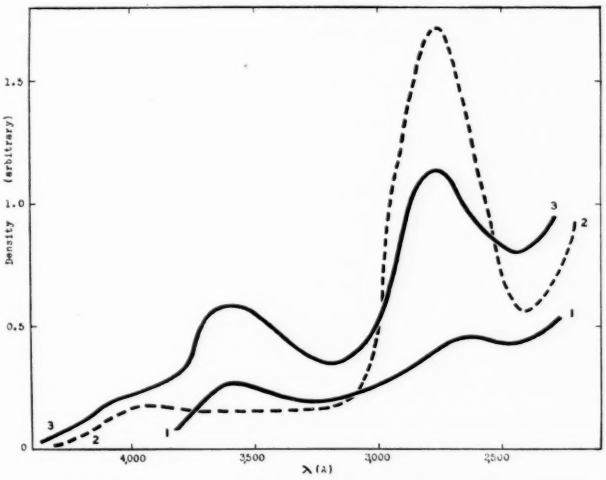

FIG. 2.—Absorption spectra of extracts in *N*/100 $NaHCO_3$ from

Curve 1. 3,4-Benzpyrene irradiated in C_6H_{12}
" 2. Benzene irradiated in C_6H_{12}
" 3. The irradiated solutions after mixing

also needed qualification. When a very dilute solution of benzene in cyclohexane (1.2 mgm. in 10 ml.) was irradiated, the absorption spectrum of the aqueous extracts initially showed only a weaker band at 2,760 A., but they also contained a well defined and stronger band at 3,600 A. (Fig. 1, curve 2). When the aqueous solutions were allowed to stand, the band at 3,600 A. diminished, whereas that at 2,760 A. increased in intensity (Fig. 1, curve 3). This result might be attributed to a reaction in which substance *B*, absorbing at 2,760 A., was produced by condensation of substance *A*, absorbing at 3,600 A., and this would allow of similar reactions between residues from irradiated benzene and from the polycyclic compounds when these were irradiated in solution in it.

Evidence that two different substances absorbing at 2,760 A. are produced was obtained when the stabilities of the products from benzpyrene in benzene, and from benzene alone, were compared. In parallel experiments benzene, and a solution of benzpyrene in benzene, were irradiated under the same conditions. The unchanged liquids were evaporated off and the residues, redissolved in cyclohexane, were re-irradiated for 30 minutes. The products were then dried and extracted in the usual way. The extract from the irradiated benzene gave no band at 2,760 A., whereas that from the benzpyrene exhibited the band at full intensity.

ORIGIN OF THE SUBSTANCE ABSORBING AT 3,600 A.

Secondary reactions similar to those suggested above could also explain the observation that the presence of benzene was necessary for the formation of a substance absorbing at 3,600 A. from naphthalene and phenanthrene, and to some extent from 3,4-benzpyrene, but not from the anthracenes or from cholanthrene. If the substance resulted from reaction of a residue from the benzene with one from the polycyclic compound, such residues might be produced simultaneously from some molecules such as anthracene, but not from smaller ones such as naphthalene. In a radical breakdown of this kind, the structures of naphthalene and phenanthrene limit the types of fission possible as compared with molecules of the anthracene type; and in so far as benzpyrene is intermediate in structural type, such an assumption might account for the lower yield obtained from benzpyrene when no benzene is added.

CONCLUSION

It is concluded that although benzene will yield water-soluble products similar to those derived from polycyclic compounds under the same conditions, when these polycyclic hydrocarbons are irradiated in the presence of benzene secondary reactions occur, which lead to the formation of compounds similar to, but not identical with, those derived from benzene alone.

ACKNOWLEDGMENT

Our thanks are again due to the Medical Research Council and, latterly, to the British Empire Cancer Campaign, for personal and expenses grants to one of us (C. B. A.) during the period when these experiments were being made.

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Effects of Ultraviolet Radiation on 3,4-Benzpyrene and Other Polycyclic Aromatic Hydrocarbons

III. Carcinogenic Activity of Aqueous Extracts from Irradiated 3,4-Benzpyrene*

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(Received for publication September 4, 1945)

INTRODUCTION

It was shown in preceding papers (2, 3) that when 3,4-benzpyrene dissolved in benzene was irradiated with monochromatic radiation of wave length 2,537 A. in the presence of oxygen, at least two water-soluble substances were included in the products and that these could be recognized, and some of their properties deduced, by spectrophotometric methods. The first, substance *A*, was characterized at pH 8 by an absorption band whose maximum intensity was at 3,600 *A*. It was labile, readily taking up atmospheric oxygen irreversibly when its aqueous solutions were allowed to stand at room temperature; but it could be stabilized by cooling them to 0° C. and storing them in an atmosphere of nitrogen. The second substance, *B*, absorbed at 2,760 A. and was more stable to oxygen, but it was destroyed by strong alkali. It reacted readily with glutathione and with other compounds containing sulfhydryl groups.

Water-soluble oxidation products of 3,4-benzpyrene are of course well known. A monohydroxy derivative is excreted as a metabolite when the hydrocarbon is fed to rats (8, 13); and a photoderivative has been described that has the power to inhibit respiratory enzymes (6, 7). None of them, however, has been reported to have any carcinogenic activity. Rideal and Schulman have shown (12) that materials very similar to, if not identical with, substances *A* and *B* are readily adsorbed onto protein monolayers, and so might be expected to pass more readily than the unadsorbed hydrocarbon through cell membranes. By analogy with hemolytic substances, which they showed to possess this property to a high degree, substances *A* and *B* might thus be expected to be biologically active. Other observations recorded in the literature, which were summarized in the introduction to Part I, seem to support this view. Since the absorption bands of *A* and *B* do not accord with those of any derivative of 3,4-benzpyrene yet recorded, a direct test of these substances for biological, and more especially for carcinogenic, action was desirable.

Preliminary investigations gave the following promising results:

1. An extract in *M*/500 sodium bicarbonate solution from irradiated benzpyrene was lethal to paramecia, and the toxicity appeared to decrease with time in parallel with the decay of intensity of the absorption band of the labile component, *A*.

2. In fibroblast cultures from embryonic chick heart grown in a medium to which aqueous extracts were added, a large number of chromosomal abnormalities appeared during mitosis, and about three times as many cells were found in prophase in the treated culture as in control cultures grown in normal medium (1).

3. One of 3 mice that were painted daily with a pure aqueous extract from irradiated benzpyrene developed a typical preneoplastic lesion on the painted area after 34 paintings; *viz.*, irregular epilation, hyperkeratosis, abnormal regeneration of hair follicles, ill-defined epidermal projections, a large increase in the number of mitotic cells, numerous abnormal mitotic figures, and leukocytic infiltration of the dermis.

In the light of these preliminary experiments, it was decided to investigate thoroughly the effect of painting the aqueous extracts from irradiated benzpyrene on the skin of mice. The results of this investigation are recorded below.

EXPERIMENTAL

Preparation of Materials

Solution I.—1.5 ml. of a 1 per cent solution of 3,4-benzpyrene in benzene were diluted to about 20 ml. with a mixture of benzene 1 part and cyclohexane 4 parts (v/v), and irradiated for 30 minutes in an open dish, area 260 sq. cm., under the conditions described in Part I (2). The remaining solvent was quickly evaporated in a current of air and the dry

* Because of the difficulties of international communication the author has not read proof of this article.

residue was extracted with 4 ml. of *M*/100 bicarbonate solution. The extract was filtered through Whatman No. 1 filter paper. Fresh material was prepared for each painting, and it was used immediately after filtration.

Solution II.—This was prepared in the same way as solution I, but without the irradiation. The extraction was done with 2 ml. of bicarbonate, and an equal volume of acetone was added to the final filtrate.

Solution III.—This also was prepared in the same way as solution I, but without irradiation. The extraction was carried out with 4 ml. of bicarbonate, and no acetone was added.

Solution IV.—This consisted of *M*/100 bicarbonate only.

Animals and Method of Treatment

The mice used came from a National Institute stock that was known to have been free from spontaneous tumors for several generations. Solutions I, III, and IV were applied daily (*i.e.*, about 5 times weekly) with a camel's-hair brush to an area about 1 cm. in diameter on the back of each animal; 0.15 ml. was rubbed in until the skin was impregnated, the fur being clipped initially and as required during the experiment. Solution II was applied daily to a similar area with a pipette; 0.3 ml. was given to allow for the dilution with acetone. The solution dried on the skin immediately.

Histological Examination

As tumors developed the animals were killed, together with an equal number of controls. The painted areas of skin were removed, fixed in Heidenhain's susa or acetic-Zenker fixatives, and serial sections were cut at 10μ and stained with hematoxylin and eosin.

TUMOR INDUCTION WITH AQUEOUS EXTRACTS FROM IRRADIATED 3,4-BENZPYRENE

If oxidation of the hydrocarbon does in fact play any part in the production of tumors when 3,4-benzpyrene is painted in sufficient quantity onto the skin of mice, the process might be pictured as involving the slow conversion of insoluble hydrocarbon at the site of painting into water-soluble substances that can penetrate into the cells of the adjacent epidermis in sufficiently high local concentration and for sufficient time to produce irreversible cytological changes. The persistence of the hydrocarbon in the painted skin would ensure that a steady concentration of water-soluble oxidation products could be maintained at least over a period of several days. In testing the solutions containing the mixed substances, *A* and *B*, no means of maintaining such steady conditions is available, and the best that can be done is to paint the mice as often as possible with the most concentrated solutions obtainable. Under these circumstances, the results do not permit any estimation of the potency of the material as a carcinogenic agent, since the time required for tumors to develop will depend both on the concentration, the absolute value of which cannot be determined until the substances have been isolated in a pure form, and on the stability of the solutions. It is possible only to decide whether or not a carcinogen is present in the experimental material.

Ten mice, from the tumor-free strain, were painted about 5 times weekly with solution I, the extract from irradiated benzpyrene. The mice underwent a sequence of slow partial epilation and subsequent slow regeneration of hair reminiscent of the more rapid changes that occur in mice painted with benzpyrene itself.

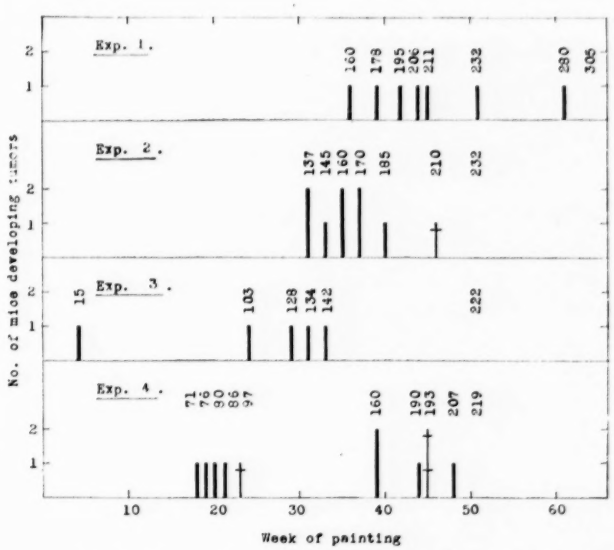

Fig. 1.—Incidence of tumors in animals painted with water-soluble products from 3,4-benzpyrene. Figures indicate number of paintings; † represent tumors in control mice.

When the experiment was terminated after 66 weeks, 7 of the animals had developed tumors on the painted areas, and histological examination revealed specific precancerous lesions in 2 others. The remaining animal showed only nonspecific dermal infiltration and slight hyperplasia. The times of incidence of the tumors are shown graphically in Fig. 1, Experiment 1. The tumor-bearing animals had at least 17 large and confluent growths among them. Histologically, these were found to be multiple keratinizing squamous cell carcinomas, among which was one of a baso-squamous cell type, and they were associated with numerous small papillomas (Figs. 2 and 3).

Since solution I probably contained minute quantities of suspended benzpyrene (see below), 10 mice from the same strain were treated concurrently with solution II, which contained the same amount of benzpyrene but no substance *A* or *B*. This control

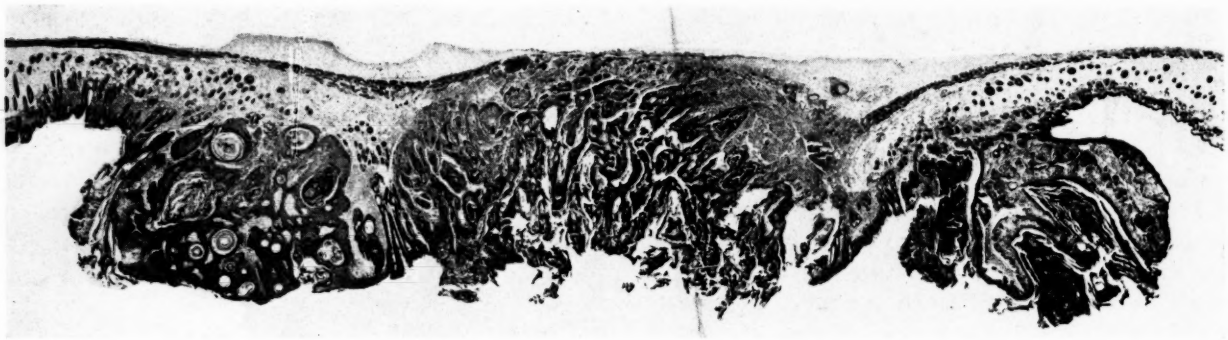

FIG. 2.—Mouse skin treated with a bicarbonate extract from irradiated 3,4-benzpyrene 206 times over a period of 44 weeks. Two papillomas flanking a squamous cell carcinoma that has invaded the panniculus carnosus. Mag. × 12.

solution was further weighted against the experiment since it contained acetone, which was added in order to facilitate the passage of any hydrocarbon present into the skin. No macroscopic changes were observed in these animals, and histological examination revealed changes in the skin of only 1 of them; *viz.*, a small area with some (nonspecific) hyperplasia and considerable mitotic activity, but with no involvement of hair follicles.

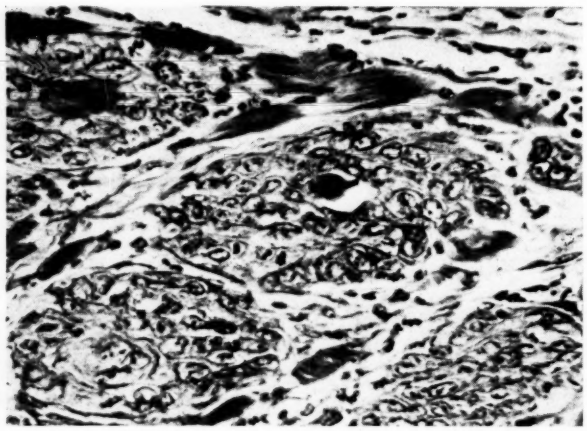

FIG. 3.—Same section of mouse skin as that shown in Fig. 1. Foci of squamous cell carcinoma inside the layer of dermal muscles. Mag. × 198.

This absence of any effect from repeated applications of small traces of benzpyrene was confirmed in a subsequent experiment, in which 10 mice were painted in the same way as those treated with solution I with a suspension of the hydrocarbon in bicarbonate (solution III). No macroscopic or microscopic changes resulted.

The results of these 3 series of paintings are set out in detail in lines i, ii, and iii of Table I. They provide evidence that the aqueous extracts from the photo-oxidized 3,4-benzpyrene contain a carcinogenically active substance that may not be the hydrocarbon itself.

TUMOR INDUCTION BY AQUEOUS EXTRACTS FROM IRRADIATED BENZPYRENE IN CONJUNCTION WITH SUBCARCINOGENIC DOSES OF PARENT HYDROCARBON

Confirmation of this conclusion was obtained in experiments in which solution I was painted on the skins of mice that had previously received either 1 or 2 paintings with subcarcinogenic doses of 3,4-benzpyrene dissolved in benzene. Three such experiments were made. In Experiment 2, 10 mice from the same tumor-free stock each received a single dose of 1.5 ml.

TABLE I: CARCINOGENIC ACTION OF EXTRACTS FROM IRRADIATED 3,4-BENZPYRENE

	Exp. no.	Treatment	No. of mice	Total No. of paintings	No. of survivors	No. of mice with tumors	No. of mice with specific precancerous lesions
i		I	10	306	10	7	2
ii	1	II	10	306	9	0	0
iii		III	10	300	8	0	0
iv	2	1BP + I	10	232	9	8	1
v		1BP + IV	10	232	10	1	0
vi	3	2BP + I	10	222	9	5	1
vii		2BP + IV	10	222	10	0	1
viii	4	1+ 1BP + I	10	219	10	8	1
ix		1 + 1BP + IV	10	219	8	3	1

Treatment: I, II, III = painting with solution I, II, III only, respectively. 1BP + I and 1BP + IV = single initial dose of benzpyrene followed by I or IV respectively. 2BP + I and 2BP + IV = two initial doses of benzpyrene with 4-day interval followed by I or IV respectively. 1 + 1BP + I and 1 + 1BP + IV = two doses of benzpyrene with 9 weeks' interval followed by I or IV respectively.
Solutions: I = bicarbonate extract from irradiated benzpyrene. II = bicarbonate extract from unirradiated benzpyrene + acetone. III = bicarbonate extract from unirradiated benzpyrene. IV = bicarbonate only.

of a 1 per cent solution. In Experiment 3, 2 such doses were given with a 4 day interval, and in Experiment 4 an interval of 9 weeks separated the 2 paintings. Daily painting with solution I was commenced on the seventh day after the first of the hydrocarbon paintings in each case, and was continued for about 50 weeks. Each experiment was controlled with an equal number of mice, which received the same treatment with benzpyrene but were painted subsequently with sodium bicarbonate (solution IV) only.

The results are set out in lines iv to ix in Table I. In Experiment 2, only 1 control animal developed a tumor. This was a large papilloma not on the painted area. There were no tumors in the controls to Experiment 3, but 3 out of 8 survivors in the controls to Experiment 4 had sebaceous gland tumors (adenomas) in the skin painted with bicarbonate. The skins of 2 other control mice in the 3 sets were found on histological examination to have specific precancerous lesions. The doses of benzpyrene as administered in Experiments 2 and 3 were thus subcarcinogenic, or threshold, whilst the administration of 2 doses of hydrocarbon with an interval of 9 weeks in Experiment 4 was perhaps too drastic.

The results with the experimental animals, however, were in striking contrast. Twenty-one out of 28 survivors in the 3 experiments had tumors, which were either squamous or basal cell carcinomas, or sarcomas; some had both, and 3 other mice had specific precancerous lesions. The incidence of these tumors is shown in Fig. 1. It is interesting that although sebaceous gland tumors occurred among the control mice in Experiment 4, no such growths were found in any of the experimental animals, among which multiple squamous cell carcinomas were most common. The sarcomas were probably caused by access of solution I to subcutaneous tissue after ulceration had begun in the superficial lesions.

DISCUSSION

There are five possible explanations of these results: (a) One or other (or both) of substances *A* and *B* is a carcinogen.[1] (b) The tumors were caused by the repeated application of minute traces of benzpyrene over an extended period of time. (c) Benzpyrene is more soluble in water in the presence of *A* and *B*, so that larger quantities were administered than those estimated in extracts from unirradiated hydrocarbon. (d) The carcinogenicity of such minute traces of benzpyrene was enhanced, cocarcinogenically, by trauma due to brushing in the solution. (e) The aqueous extracts from irradiated benzpyrene contained an unidentified carcinogen, which was neither substance *A* nor *B* nor suspended benzpyrene.

Of these possibilities, (b) and (d) were excluded by the double control experiments made in parallel with Experiment 1. Explanation (c) is invalidated by the results of estimations of the free benzpyrene in solution I, by the method described in Part I (2), which gave a maximum concentration of 2.73×10^{-6} gm. per ml. as compared with 2.73×10^{-6} gm. per ml. for the control solution II. The two values may be considered to be identical within experimental error.

If it is assumed (i) that every extraction of the irradiated hydrocarbon took up the maximum concentration of benzpyrene, and (ii) that all the benzpyrene in any dose penetrated into the mouse skin during painting, which is unlikely with the aqueous solution, I, but more probable with the acetone: water control solution, II, the possible quantity of suspended hydrocarbon in an individual dose would be about 4×10^{-7} gm. The aggregate quantity that could have been administered in this way to any animal in the control to Experiment 1 was 8.6×10^{-5} gm. No tumors were observed. Half the experimental animals, however, had developed tumors after 206 paintings, which could have contained only 8.2×10^{-5} gm. In Experiment 2 the suspended benzpyrene contained in the 232 paintings with solution I amounted to about 6 per cent of the initial dose, while in Experiments 3 and 4 it was considerably less. The presence of these small quantities of hydrocarbon, in the light of the present results, seems therefore to have little significance.

Examination of the figure shows a difference in the rates of appearance of tumors in Experiments 2, 3, and 4 (taken together) and in Experiment 1. Statistical analysis [2] shows that this difference is nearly three times greater than its standard deviation, and is therefore significant. Auxiliary painting with benzpyrene thus resulted in an acceleration of tumor formation. If the benzpyrene so applied is considered to be a precarcinogen, in the sense defined by Berenblum (4, 5), the active substance in solution I might be considered to have, in particular, epicarcinogenic properties.

In the light of the experiments of Crabtree (9, 10, 11), who showed that substances that react with cysteine or with glutathione and inhibit cell glycolysis also retard the rate of tumor formation by chemical carcinogens, it is unlikely that substance *B* is a carcinogen. Moreover, it is conceivable that positive action by *A* is partially inhibited by the negative action of *B*. This point can only be decided when more efficient methods of separating *A* from *B* have been devised.

[1] A sixth possibility, namely that the extracts contain a cocarcinogen, is being investigated in experiments now in progress. Results so far obtained neither support nor exclude it.

[2] I am indebted to Dr. D. E. Lea, of this laboratory, for the calculation.

It may be concluded that the carcinogenic action of the aqueous extract from photo-oxidized 3,4-benzpyrene was due to substance *A*, unless there was another carcinogenic substance present (not the parent hydrocarbon) as yet unidentified. The results described lend a certain support to the thesis that oxidation processes play some part in the production of tumors by carcinogenic hydrocarbons.

SUMMARY

It is suggested that aqueous extracts from photo-oxidized 3,4-benzpyrene contain a carcinogenic substance, which may be a labile compound whose presence has been established by means of its absorption spectrum.

ACKNOWLEDGMENT

My thanks are due to the Medical Research Council and to The British Empire Cancer Campaign for personal and expenses grants in separate periods during which this work was being done. I am greatly indebted to Dr. A. Glücksmann, of this laboratory, for making all the histological examinations and for much helpful advice, and to Mr. G. Lennie and Mr. H. G. Hignell for technical assistance.

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Committee on Growth of the National Research Council

The appointment of a "Committee on Growth," with membership designed to be broadly representative of the fields concerned in cancer research, both basic and clinical, has already been announced by the National Research Council of the National Academy of Sciences. The Committee was created, within the Division of Medical Sciences of the Council, as a result of action by the American Cancer Society designating the Academy as its scientific adviser for research.

The Committee wishes to call the attention of interested investigators to the general outline of endeavor which it proposes to foster and the general principles by which it will be guided. The Committee accepts the interpretation of its field of interest as including reliance on, contact with and support of research in the basic sciences bearing broadly on the whole phenomenon of growth.

The Committee has adopted the following major principles by which, in so far as possible, it will be guided in its sponsorship of research and training programs:

(a) Desirability of long-term grants to projects of major importance.
(b) Grants, where possible, of such magnitude as to permit individual investigators to appoint associates for long-term training periods.
(c) Granting of fellowships to institutions for training of workers to acquire new technics and wider experience.
(d) Maintenance of continuing individual contact with workers in field.
(e) Provision, on a participating basis, for continuing economic security for professional workers.
(f) Liberal attitude toward the investigator's work, his publication and reports.

To assist it in the fulfillment of its advisory functions the Committee, on its part, will make free use of either *ad hoc* or standing subcommittees in specific fields of interest. Furthermore, it proposes to arrange conferences of competent groups for discussion of problems, for interchange of reports, etc.; make surveys to analyze problems or to determine progress in areas of special interest pertaining to cancer; evaluate, through study by subcommittees and by the main committee, basic and clinical research undertakings, and submit recommendations for support to the American Cancer Society; initiate and plan broad or specific programs of basic and clinical research, through activities of the subcommittees and main committee, and secure the cooperative efforts of investigators in the general undertakings.

The Committee has established a central office in the Washington headquarters of the Council where information on all phases of cancer research will be assembled and from which reports may be distributed to interested investigators.

Many members of the Committee have participated intensively in the broad programs of research conducted under the pressure of war. It is both the hope and the sanguine expectation of the Committee that the fruitful pattern of cooperative investigations so successfully established during the war years, can now be carried on, modified and tempered to existing needs, into the continuing war against disease.

Membership of the Committee, as now constituted, includes the following:

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Dr. Florence R. Sabin, *Secretary*
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William Cramer

(1878–1945)

Whom the gods love die young. If this poignant phrase refers to years only it does not apply to William Cramer, for he was just over sixty-seven at the time of his death. But if it means that these favorites of the gods retain a youthful outlook until the end, nothing ever said of him was more true. His restless, inquiring mind ranged over the fields, to him always the Elysian fields, of biochemistry, physiology, and cancer research; the nervous system; metabolism, including that of the cancer cell; growth; the coagulation of blood; surface tension; the lipids; fever; infections; vitamins; hormones; cell structure; the parasitic etiology of cancer; diet; statistics; radiotherapy; resistance and susceptibility to cancer; the virus question; and carcinogenesis, the problem that filled so happily the evening of his life.

By reason of his early training in physiology and biochemistry and his subsequent position on the scientific staff of the Imperial Cancer Research Fund, first directed by Bashford, later by Murray, and then by Gye, he was amply equipped to conduct almost any sort of investigation into malignant neoplastic disease. Add to these advantages an acute, perceptive mind and an admirable critical sense if an explanation be desired for his rapid rise to a commanding position among the oncologists of the world.

During the London period he was accounted one of Europe's most competent biochemists, and the generosity with which he shared his great store of precise information with colleagues who were less fortunately endowed, or less well equipped, was often the subject of remark.

Cramer was born in Brandenburg, Prussia, on June 2, 1878, to Siegmund and Olga Cramer, nee Harff, and died suddenly of coronary occlusion while on vacation in Denver, Colorado, on August 10, 1945.

He received the degree of Ph.D. from the University of Berlin in 1900, and in the same year was appointed Research Chemist there. His D.Sc. was conferred in 1906 by the University of Edinburgh, and his M.R.C.S. and L.R.C.P. by University College, London, in 1917, two years after he had acquired British citizenship. From 1903 to 1905 he was a junior member of the staff of the Imperial Cancer Research Fund, London, and a senior member from 1915 until his retirement in September 1939; the intervening years were spent as Lecturer on Chemical Physiology at the University of Edinburgh.

Cramer was at one time on the committee responsible for Acta of the International Union against Cancer, and a member of the Editorial Committee of The Cancer Review, a journal that had the courage to replace the customary tepid and colorless abstract with critical, and sometimes even mordant, ones. He was a delegate of the British Government at the international cancer conferences in Madrid, 1933; in Brussels, 1936; and in Atlantic City, 1939. In 1940 he was invited to deliver the Middleton Goldsmith Lecture of the New York Pathological Society.

He was the author of two books—one on practical chemical physiology and one on fever; of chapters in three books—on the physiology of reproduction, on cancer research, and on histological technic respectively; and author or co-author of well over a hundred monographs.

Soon after arriving in the United States, in 1939, he received an appointment as Research Associate at the Barnard Free Skin and Cancer Hospital, St. Louis, where he remained, actively and fruitfully engaged, until his death.

In person William Cramer was spare and of average height, with the slight stoop so often ascribed to the student, and the immediate impression that here was a scholar was reinforced by the concave lenses that his myopia demanded. As was to have been expected, his pastimes ran to the intellectual rather than to the athletic; to music, to literature, to the graphic arts, rather than to golf and tennis, though he was fond of riding—a reversion, perhaps, to the days of his youth, when he passed his year of military training in a cavalry regiment. A charming host in his own home, as the writer can testify, he preferred in his leisure hours to discuss the opera, the latest art exhibit, current events, and other topics of general interest rather than the minute details of his calling.

Though a member of a group whose energies are focussed on one single problem, he must have been a little skeptical of coordinated cancer research on the grand scale that is being proposed today. For on one occasion, when its advantages had been suggested in conversation, he replied in his quiet way: "Yes, but the groups of forty or fifty chemists that industry turns loose on a problem know what they are looking for."

Besides a host of friends and admiring colleagues he leaves a widow, the former Belle Klauber, to whom he was married in 1906, and two sons: Ian William David, born in 1913, and Michael William Valentine, born in 1924.

It is frequently said of a man that his place cannot be filled. Often enough an agreeable fiction, this applies with special cogency to William Cramer, for his broad training and his natural ability together made him a unique figure in his chosen field.

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Cramer, W. The Riddle of Cancer. C. Oberling (Book Review). Cancer Research, **4**:669-672.

Cramer, W., and Simpson, W. L. Mast Cells in Experimental Skin Carcinogenesis. Cancer Research, **4**:601-616.

1945. Simpson, W. L., Carruthers, C., and Cramer, W. Loss of Carcinogenic Activity when Methylcholanthrene Is Dissolved in Anhydrous Lanolin. Cancer Research, **5**:1-4.

Simpson, W. L., and Cramer, W. Sensitization of Skin by Carcinogenically Inactive Methylcholanthrene to Subsequent Carcinogenesis. Cancer Research, **5**:5-10.

Simpson, W. L., and Cramer, W. Fluorescence Studies of Carcinogens in Skin. II. Mouse Skin after Single and Multiple Applications of 20-Methylcholanthrene. Cancer Research, **5**:449-463.

William H. Woglom

Abstracts

Reports of Research

The Effect of Purines on Polycyclic Aromatic Hydrocarbons. WEIL-MALHERBE, H. *Biochem. J.*, **39**:xxxvi. 1945.

The effect of caffeine on the solubility in water of 3,4-benzpyrene discovered by Brock, Druckrey, and Hamperl (*Arch. exper. Path. u. Pharmakol.*, **189**:709. 1938) has been further studied and extended to include other purines and hydrocarbons. The solvent effect increases with the number of substitutions by oxygen of the positions 2, 6, and 8, and with the number of N-methyl groups; thus hypoxanthine stands at one end of the scale and tetramethyl-uric acid at the other. 3,7-Dimethylpurines have more effect than have 1,3-dimethylpurines. The nucleosides, adenosine and guanosine, also show a solvent effect. The solvent power of the purines having acidic properties is greatly reduced in alkaline solution. The solvent power of caffeine and tetramethyl-uric acid, which have no acidic properties, is unaffected by alkali. The quenching effect of purines on the fluorescence of polycyclic aromatic hydrocarbons was also studied.—E. L. K.

The Effects of Methylation on the Activity of Nitrogenous Carcinogens. 1. *p*-Aminoazobenzene in the Rat. KIRBY, A. H. M. *Biochem. J.*, **39**:xxxiii. 1945.

p-Aminoazobenzene was fed to 16 rats; 1 died (13 months) with small hepatomas and no cirrhosis; 2 died (17 months) with massive hepatoma, cholangioma, and angioma. One showed secondary hepatoma in the mesentery, the other primary lung adenoma. Others dying later showed small hepatomas; cirrhosis was not constant.—E. L. K.

The Effects of Methylation on the Activity of Nitrogenous Carcinogens. 2. Azo-dyes and N-methyldibenzcarbazole in Mice. KIRBY, A. H. M. *Biochem. J.*, **39**:xxxiii-xxxiv. 1945.

Liver tumors were not induced by subcutaneous injection of *p*-aminoazobenzene in stock mice. 2′-Amino-4,5′-azotoluene produced no tumors when injected into stock mice; local massive necrosis of the liver was frequent. Butter-yellow is much more carcinogenic than is *o*-aminoazotoluene when fed to rats. The reverse is true for mice; hence one theory, *e.g.*, the split product theory, cannot account for both sets of phenomena. N-Methyl-3,4,5,6-dibenzcarbazole applied to the skin or by subcutaneous injection to stock mice has a slower carcinogenic action (papillomas, sarcomas) than the parent nonmethylated compound and does not produce tumors of the liver.—E. L. K.

Action des agents cancérigènes chimiques sur les levures. Action de l'arsenic. [The Effect of Chemical Carcinogens on Yeast Cells. The Effect of Arsenic.] BERAUD, P. [Pasteur Inst., Paris, France] *Ann. Inst. Pasteur*, **69**:230-237; 275-286; 349-357. 1943.

Sodium arsenite and arsenate added in sublethal concentrations to Springer's yeast cultures give rise to the formation of yeast cells of different shape, size, and weight. The treated cells, when recultured on ordinary media, multiply more rapidly, their dry weight is lower, and their radiosensitivity higher. After arsenate, their glycogen content is lower, but it is higher after arsenite. All these new characters disappear after a few transfers on ordinary media. The oxygen uptake and fermentation activity of Springer's yeast grown in the presence of sodium arsenate are accentuated; in the presence of sodium arsenite they are reduced. In both cases the rate of cell division is lowered.—C. A.

The Carcinogenic Activity of Irradiated and Non-Irradiated Sterols and Related Substances. PIZZOLATO, P., and BEARD, H. H. [Charity Hosp., and Louisiana State Univ. Sch. of Med., New Orleans, La.] *Exper. Med. & Surg.*, **3**:95-99; 297-298. 1945.

Cholesterol, ergosterol, desoxycholic acid, dehydrodesoxycholic acid, bile salts, and gallstones (from a patient dying of rheumatic fever), in irradiated (104 hours 20 cm. from G-E Sunlight Lamp, Type S-1) and nonirradiated forms, were injected intraperitoneally into rats, in dosage of 10 mgm. monthly for 6 months; all animals surviving at the end of 10 months received 1 further injection of 100 mgm. Each substance was tested on 20 to 75 rats, and the total number of animals used was 389. Four animals developed malignant tumors: a fibrosarcoma after irradiated cholesterol administration, an anaplastic sarcoma after cholesterol, an anaplastic sarcoma after dehydrodesoxycholic acid, and a keratinizing squamous cell sarcoma after irradiated ergosterol. The first 3 tumors were attached to the peritoneum, the fourth was found between the ear and nose. No spontaneous tumors have arisen in this rat colony for 17 years.—M. H. P.

Mechanism of the Feulgen Reaction. CARR, J. G. [Inst. of Animal Genet., Univ. of Edinburgh, Edinburgh, Scotland] *Nature, London*, **156**:143-144. 1945.

As a result of staining experiments upon normal, malignant, and embryonic tissues and such material as newsprint, filter paper, cotton wool, and alumina, the author concludes "that neither the presence nor the location of the stain bears any relation to the presence or absence of either of the two nucleic acids but can be merely a reflexion of the absorbing power of the chromosomes."—E. L. K.

The Action of Carcinogenic Compounds and Related Substances on Enzymes. FEIGENBAUM, J. [Hebrew Univ., Jerusalem, Palestine] *Exper. Med. & Surg.*, **3**:28-34. 1945.

See *Exper. Med. & Surg.*, **2**:304. 1944; abstr. in *Cancer Research*, **5**:250. 1945.—M. H. P.

Respiration and Anaerobic Glycolysis of Livers and Hepatomas in Mice Fed Orthoaminoazotoluene. ELTSINA, N. V. *Am. Rev. Soviet Med.*, **2**:500-507. 1945.

The experiments deal with the effect of carcinogens upon metabolism of tissues undergoing malignant change.

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

One per cent *o*-aminoazotoluene in benzene was painted on mice. Respiration and anaerobic glycolysis of liver slices were followed until hepatomas developed. A lowered rate of oxidation was found only in hepatomas. In one series of mice, painted for 5 months, the regenerating liver showed increased oxidation. Anaerobic glycolysis of liver slices after the mice were painted and before hepatomas appeared showed no change from the normal. Hepatomas, like other malignant tumors, show a high increase in anaerobic glycolysis. Neither oxidative processes nor anaerobic glycolysis show any change in the precancerous period. The transformation of the normal cell into cancer evidently does not depend on the metabolic changes reported.—J. H.

Nutrition and Growth and Their Influence on Longevity in Rats. Saxton, J. A., Jr. [Cornell Univ. Med. Coll., New York, and Sch. of Nutrition, Cornell Univ., Ithaca, N. Y.] *Biol. Symposia,* **11**:177-196. 1945.

A summary of experiments conducted by C. M. McCay, J. A. Saxton, Jr., and their associates. The more recent studies, which will be described in greater detail elsewhere, have been concerned with the principal diseases affecting the life span of the rats, especially tumors, chronic nephrosis, and chronic pneumonia with bronchiectasis. These and other diseases proved less frequent, and appeared later, in rats whose growth was retarded by calorically restricted diets than in control rats. The effects were particularly striking with regard to spontaneous pulmonary lymphosarcoma, a chromophobe adenoma-like growth in the pituitary, and chronic nephrosis.—M. H. P.

De la radiosensibilité des tumeurs malignes. [The Radiosensitivity of Malignant Tumors.] Grynkraut, B. *Presse méd.,* **48**:899-900. 1940.

Lethal doses of x-rays given to rats bearing the Jensen sarcoma have no effect on the ability of the tumor, removed 1 hour after irradiation, to grow as transplants in other rats, but the growth of transplants taken 2, 48, and 72 hours after is proportionately impaired. On the other hand Jensen sarcoma transplants in rats having received sublethal doses of x-rays 3 days previously give rise to tumors smaller than usual. The author concludes that substances in the organism necessary for cancerous growth are radiosensitive.—C. A.

Susceptibility of Young and of Adult Mice to the Mammary-Tumor Agent. Andervont, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **5**:397-401. 1945.

An attempt was made to transmit the milk influence to young susceptible $(I \times C3H)F_1$ females by cutaneous transplantation of a mammary carcinoma with subsequent surgical removal. Of 23 mice that grew the transplant, only 1 developed a spontaneous mammary cancer later in life.

To test the relative susceptibility of mature and young mice, $(I \times C3H)F_1$ females were injected with a tumor extract of known potency after each had had one litter and was approximately 4 months of age. After injection they were mated to C3H males, and the young resulting from this backcross were then nursed by their mothers and were themselves subsequently bred. Of the 25 F_1 hybrid mothers, only 2 developed mammary carcinoma while, of their 115 backcross daughters, 25 developed mammary tumors. An analysis of the backcross daughters developing tumors showed all of them to be offspring of seven F_1 mothers that had raised a total of 40 young, whereas no tumors developed in 75 daughters raised by the other 14 females.

The same experiment was repeated using I instead of $(I \times C3H)F_1$ females. In this case, however, neither the I mice nor their F_1 hybrids developed tumors. These results would indicate that young mice are more susceptible to the milk influence than are mature ones, and that, while the influence was transmitted by some of the cancer-susceptible hybrid mothers, it was transmitted by none of the resistant I females.—R. A. H.

Cancer and the Process of Ageing. Loeb, L. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Biol. Symposia,* **11**: 197-216. 1945.

A review and discussion, with special reference to the author's studies on mammary carcinoma. The main cause of the increased incidence of cancer in older individuals apparently consists in the actions of stimuli that accumulate with advancing age. The importance of the genetic constitution of the tissues affected in determining responsiveness to such stimuli is emphasized.—M. H. P.

Influence of Age on the Growth of Lymphomas. Nettleship, A. [Univ. of Oklahoma, Oklahoma City, Okla.] *Am. J. Path.,* **21**:147-165. 1945.

Four transplantable lymphomas were studied in animals of different ages. With regard to local lymphomas, the gain in weight during tumor growth was accounted for in the young animals by gain in the normal tissues (one-half), and also in the tumor. In older animals the gain in weight could be accounted for only by the amount of neoplastic tissue grown (the animal's tissues lost weight). In one instance the average tumor weight of the older age group was greater, in another instance slightly less, than that of the younger age group.

It was demonstrated with the malignant thymoma that these neoplasms increased in mass one-half as rapidly in young animals as did normal young growing tissues. All of the local tumors differed from normal tissues in their ability to continue this growth unchanged beyond the slowing down of normal tissues.

No significant difference in mortality could be demonstrated in animals of different age groups when inoculated with a generalized lymphoma.—Author's summary. (J. G. K.)

Growth of a Mouse Lymphoma Compared to Normal Tissue Growth. Nettleship, A. [Univ. of Oklahoma, Oklahoma City, Okla.] *Am. J. Path.,* **21**:167-169. 1945.

Young mice which grew transplantable lymphomas accumulated approximately the same amount of tissue as their litter-mate controls, and the rate of accumulation was similar. One-half of the total gain in weight in the tumor-bearing animals was in the tumor. Older animals which bore tumors grew more tissue than their litter-mate controls. Their tumor weights could account for almost all of their weight increase.

Even in young animals, the total neoplastic mass ac-

cumulated was never greater in any time period than that grown by heterogeneous tissues in litter-mate controls. The actual growth of the protoplasm in this lymphoma appears to be approximately one-half that of heterogeneous normal tissues.—Author's summary. (J. G. K.)

Regression Produced in the Murphy Lymphosarcoma by the Injection of Heterologous Antibodies. Nettleship, A. [Univ. of Oklahoma, Oklahoma City, Okla.] *Am. J. Path.*, **21**:527-541. 1945.

Antigens made from various fractions of the rat lymphosarcoma tissue were injected repeatedly into rabbits with the aim of producing anti-lymphosarcoma serum. The chemical fraction found most useful as an antigen was that precipitated by acidification to pH 4.2 with acetic acid. By injecting sera prepared in this way into rats implanted a few days before with the lymphosarcoma, the author was able to bring about retardation in the rate of growth of the lymphosarcoma and an increase in the number of regressions, the incidence of regressions being 10% in 83 control animals, 28% in 75 serum-treated animals, and 54% in 22 animals treated with large amounts of sera that had been produced by prolonged massive immunizations and that contained high titers of antibody as determined by precipitin reactions. It proved essential to inject large quantities of the potent antisera within a few days after implantation of the tumor cells; if either dosage or titer was low there were no instances of regression, and none occurred in animals receiving antisera after the tumors had grown for a considerable period. The serum caused induration of the subcutaneous tissue at the site of inoculation, and 2 animals died from what appeared to the author to be sensitivity to the serum. Almost all animals that had received maximal dosage of antibodies showed some hyperplasia of the spleen and bone marrow.

Eight photomicrographs are reproduced that show tumors in serum-treated animals; necrosis of tumor cells and peri-tumor fibrosis are conspicuous.—J. G. K.

Heterologous Transplants of Human Carcinoma of Prostate. I. Is Human Cancer of the Prostate Gland Autonomous? Masina, M. H. [Yale Univ. Sch. of Med., New Haven, Conn.] *J. Urol.*, **53**:257-262. 1945.

Histologically proved carcinoma from 3 human prostates was transplanted into the anterior chamber of the guinea pig eye. Androgens were given to the guinea pigs in the hope of stimulating growth. None of the transplants survived for more than 30 days.—V. F. M.

A Possible Explanation of Conflicting Results in Experiments with Transplanted Tumors. Connell, H. C., and Munro, L. A. [Hendry Connell Research Foundation, Kingston, Canada] *Canad. M. A. J.*, **53**:162-167. 1945.

In transplantation experiments with Belough sarcoma in mice, by inoculation of tumor cell suspensions, the number of takes, regressions, and survivals varied with the dosage of inoculum. It is concluded that in experiments designed to demonstrate the effect of treatment on tumor-bearing animals, inoculation with standardized cell suspension is to be preferred to the less quantitative introduction of tumor fragments by the use of trochar or canula.—M. E. H.

Malignant Lymphoma (So-Called Leukemia) in Dogs. Bloom, F., and Meyer, L. M. [Flushing, N. Y., and Kings Co. Hosp., Brooklyn, N. Y.] *Am. J. Path.*, **21**:683-715. 1945.

In a group of 10,000 dogs, 20 cases of malignant lymphoma were encountered. In these cases, the lymph nodes, spleen, and liver were usually enlarged, and there were diffuse and nodular cellular infiltrations of these organs, and of the bone marrow, adrenals, lungs, kidneys, tonsils, Peyer's patches, and other tissues as well. On a cytologic basis the predominant cellular types were classified into 4 groups: lymphoblastic, lymphosarcoma cell, mixed cell, and lymphocytic. The term leukemia is deemed inadmissible for the canine disease, since the peripheral blood showed only anemia and usually a polymorphonuclear leukocytosis.—J. G. K.

Les enseignements tirés de l'étude expérimentale des leucémies et des tumeurs du système hematopoiétique. [Lessons That May Be Drawn from the Experimental Work on Leukemia and Tumors of the Hemopoietic System.] Roussy, G., and Guérin, M. [Cancer Inst., Paris, France] *Presse méd.*, **50**:445-446. 1942.

A review. All experimental data indicate that leukemia may be grouped with cancer.—C. A.

Recent Advances in Cancer Research. Lewisohn, R. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**: 464-497. 1945.

This discussion is concerned with 3 topics: (1) Recent work on the aromatic carcinogenic hydrocarbons. (2) The relationship of sex hormones to carcinogenesis. (3) A brief report of the work of the Cancer Research Laboratory of Mt. Sinai Hospital, dealing with inhibitory effects of spleen extract, yeast extract, various diets, grains, inositol, folic acid, and xanthopterin upon transplanted and spontaneous malignant tumors in mice.—A. Cnl.

Joint Discussion on Cancer, by the Biochemical Society with the Pathological Society of Great Britain and Ireland, December 16, 1944.

Biochem. J., **39**:i-x. 1945

Oestrogens and Malignant Disease

Synthetic Oestrogens in Cancer. Dodds, E. C.

The estrogens have actions, *e.g.* upon the pituitary, besides that of producing estrus, and hence the name "gynaecogen" has been proposed for them. They can be classified as: (1) natural estrogens: estrone, estradiol, estriol, and their simple esters; (2) modified natural estrogens, *e.g.* ethinyl estradiol; (3) synthetic estrogens: (a) derivatives of normal hexane: hexestrol, stilbestrol, dienestrol, and (b) derivatives of ethylene: triphenylethylene and its derivatives.

(3a) are more active than (1) while (3b) are weaker. (3a) are, unlike (1), highly active when given by mouth. These compounds have no definite effect on the growth rate, take, or fate of transplanted animal tumors. When estrogens are used in the treatment of carcinoma of the prostate "the greater majority of cases show complete relief of symptoms, and can be maintained symptom-

free for long periods. The word 'cure' must not, of course, be used, but the relief of suffering is of the greatest importance. This is the first time that any form of cancer has been favourably affected by a medicine given by mouth." The mode of action may be by inhibition of testosterone secretion by the testis, or of gonadotrophin by the pituitary, or by direct action on the malignant growth. "It is not yet possible to assign the relative importance to these three possible factors, but the fact that compounds of such widely differing chemical structure as oestradiol and stilboestrol are both capable of causing symptomatic relief, points to the activity lying in their oestrogenic activity rather than their chemical structure."

Inhibition of Growth by Amino-*s*-Diarylethylenes. Haddow, A., Harris, R. J. C., and Kon, G. A. R.

Growth-inhibitory action towards the Walker tumor discovered in 9-(*p*-dimethylaminobenzal)-fluorene suggested tests on basically substituted stilbenes. 4-Dimethylaminostilbene retarded body growth and tumor growth (Walker carcinoma) in the rat, but was toxic and less effectual towards spontaneous and grafted mouse tumors. "Inhibitory activity in the rat is retained when the dialkylamino group is transferred from the *para-* to the *ortho-* position, but is absent in *meta*-dimethylaminostilbene. Activity is annulled by *pp'*-disubstitution, by hydrogenation or extension of the ethylene chain, and by alkyl substitution of the ethylene hydrogen atoms." Reduction of toxicity was shown by the inhibitory compounds 4-dimethylamino-2'-methylstilbene and α-(4-dimethylaminophenyl)-β-(α-naphthyl)-ethylene, while the corresponding β-naphthyl compound is almost inactive. These new compounds are not estrogens; they are under test for carcinogenic activity. Amounts of 4-dimethylaminostilbene that arrest body growth do not increase the output of neutral sulfur.

The Evolution of Mouse Mammary Cancer; the Roles of Milk Factor and Oestrogen. Bonser, G. M.

Mouse mammary cancer arises as (1) most commonly, intra-acinous carcinoma, or (2) malignant acinar proliferation, or (3) intraduct carcinoma. By addition of the milk factor to resistant males and subsequent estrogen treatment, comparisons with normal mice were made. "Fully developed mammary cancer occurred only in mice with milk factor. . . . . Oestrogen causes growth of breast tissue from a simple vestigial tube to a complicated system of ducts and hyperplastic acini. Unless the lining cells are prepared or sensitized by the previous addition of milk factor, the process does not proceed to malignancy. . . . ."

"Intraduct carcinoma is the common first cancerous change in the human breast, the ducts affected being terminal. Intra-acinous carcinoma occurs much more rarely. This raises the question as to whether a milk factor exists in the human and, if it does exist, whether it is of different kind, acting upon the ducts."

The Differentiation between Prostatic Phosphatase and other Acid Phosphatases in Pathological Human Sera. Herbert, F. K.

See *Cancer Research*, **5**:379. 1945.

Plasma Phosphatase in the Diagnosis of Malignancy. King, E. J., and Delory, G. E.

The units of the plasma phosphatases are described. Acid phosphatase is not present in the prostate until puberty. Plasma acid phosphatase is normal in amount in cases of prostatic cancer without bony metastases; when such metastases are present the amount is raised (highest value observed 108 units; normal range 1 to 5 units). "Treatment with stilboestrol has nearly always caused a return of the acid-phosphatase to normal concurrently with improvement of the clinical condition of the patient." See *Cancer Research*, **5**:320. 1945.

The Clinical Use of Synthetic Oestrogens in Carcinoma of the Prostate. Riches, E. W.

"The treatment of carcinoma of the prostate demands more than the giving of stilboestrol; without adequate investigation and control the results are often unsatisfactory." Predominant symptoms may be (A) urinary or (B) metastatic. In group A endoscopic resection provides a biopsy, and gives relief more rapidly than does stilbestrol; in group B resection may not be required. In all cases stilbestrol is started as soon as the serum acid phosphatase is known; dose 1 mgm. increased to 5 mgm. 3 times daily; maintenance dose 1 to 10 mgm. daily. "The results to be expected in over 90% of cases are a gain of weight with an improvement in general condition and urinary symptoms; the prostate becomes softer; enlarged lymphatic glands diminish in size and lymphatic oedema disappears. The pain from osseous metastases may disappear. . . . . If the maintenance dose is inadequate the general condition deteriorates and the acid phosphatase rises again." Side effects are enlargement of breasts, pigmentation of nipples and midscrotal line (patients who pigment deeply respond well), decrease in size of penis and testis, and impotence. "The most striking results are seen in the most advanced cases."

Viruses in Relation to Cancer with Special Reference to the Milk Factor

Gye, W. E. The cancer cell carries its own stimulus to growth, which is inexhaustible. "The immediate practical problem is to reconcile filtrability of some tumours with non-filtrability of others," for tumors that can, and cannot, be propagated by filtrate are the same kind of pathological growth. Bittner's milk factor can act "only under the most narrowly stringent conditions," and this behavior is characteristic of cancer viruses.

McIntosh, J. The Rous tumor agent has antigenic properties comparable to those of bacteria and other viruses. Five filtrable tumors have been produced in fowls by means of tar in lard. The observations of Rous on the acceleration of the Shope papilloma by tar have been confirmed; tar appears to act in this case, and in the induction of fowl tumors, in the same way as do estrogens in relation to the milk factor.

Gordon, M. Encouraging results were obtained by treating Hodgkin's disease by a combination of elementary bodies from the lesions with their antibodies, and a similar treatment of sarcomas is under trial. Filtration

reduces the potency of viruses. The virus theory of cancer is confirmed by: (a) the presence of inclusions in acute cases of carcinoma, and (b) the relative immunity of the small intestine to cancer, since the contents contain a viricidal factor secreted by the pancreas. This action ceases at the large intestine.

VAN ROOYEN, C. E. Smear preparations from lymphadenomatous glands showed occasionally extremely scanty particles resembling elementary bodies.

CARR, J. G. The incidence of spontaneous tumors in ordinary flocks of fowls may be as high as 30%, "and it is considered that most of these are caused by viruses." Hence it is not surprising that such birds are 98 to 100% susceptible to the Rous No. 1 sarcoma virus. A brown Leghorn flock has been raised that has an abnormally low tumor incidence—less than 0.1%, but is equally sensitive to Rous No. 1 virus. Another line of fowls was bred, of which only 10% were susceptible to this virus.

PEACOCK, P. R. The 57 chemically induced fowl sarcomas produced by the author resemble the analogous mammalian tumors in all demonstrable respects, and differ from the virus sarcomas of fowls in that they contain no recoverable virus. As most of the virus tumors originated spontaneously, the initial relationship of the virus to the tumor is not known; it might have been an extrinsic agent or a product of the tumor. The existence of several types of virus tumors, and even the discovery of further examples in several species of animals, is no logical reason for postulating a viral cause for all tumors against the balance of evidence. The virus induced sarcomas differ from those chemically induced in their tendency to undergo spontaneous cure. Carr has shown that this tendency may have a genetic basis.

"Histologically, these tumors are sarcomas, yet spontaneous cure is certainly not a characteristic feature of sarcoma in man. This calls in question the value of histological classification as a guide to etiology. . . . .

"The desire to find a specific cause of cancer probably arises from the idea of cancer as a single disease rather than as a special type of pathological tissue reaction. The weight of evidence available at the present time points to multiple causes of cancer, as in the case of most other types of lesion and tissue reaction."

Papers of the Joint Discussion abstracted by E. L. K.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

THERAPY—GENERAL

Cancer et système lymphatique. Faut-il respecter les tissus sains? [Cancer and the Lymphatic System. Should Normal Tissues Be Spared?] ROUX-BERGER, J.-L. [Tenon Hosp., and Curie Foundation, Paris, France] *Presse méd.*, **48**:617-620. 1940.

A discussion. Experimental and clinical observations indicate that normal lymphatic tissue has no restraint on the growth of cancer cells. The surgical treatment of cancer should never be conservative, and even normal lymph ducts and nodes usually invaded by the type of cancer treated should be removed.—C. A.

Radiation and Neurosurgery in Advanced Cancer. COOPER, G., JR., and ARCHER, V. W. [Univ. of Virginia Hosp., University, Va.] *Virginia M. Monthly,* **72**:108-113. 1945.

The case of late cancer can seldom be handled to best advantage without the close cooperation of a number of physicians. Many patients can be relieved of pain by radiation; an occasional cancer can be arrested indefinitely; some can be controlled for years and months. Radiation produces bone regeneration in many cases of destructive bone metastases, and orthopedic support may prevent pathological fractures. Persistent pain is an indication for radiation in patients with known cancer. Radiation may be a valuable supplement to orchidectomy in metastatic carcinoma of the prostate. The ability of the neurosurgeon to relieve pain in many cases of late cancer should be kept constantly in mind. Ten illustrative case histories are presented.—M. E. H.

RADIATION

Quelques appareillages accessoires de la curiethérapie. [Accessory Devices for Use in Radium Therapy.] WICKHAM, Y.-L. [Cancer Inst., Univ. of Paris, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **29**:88-89. 1940.

A description of devices for the protection of normal tissues in radium therapy of cancer of the cervix and the mouth.—G. H. H.

Le cancer expérimental provoqué par le dioxyde de thorium. [Experimental Cancer Produced by Thorium Dioxide.] ROUSSY, G., and GUÉRIN, M. [Cancer Inst., Paris, France] *Presse méd.,* **49**:761-763. 1941.

A review of experimental work on sarcoma produced in animals by thorium dioxide (Thorotrast) leads the authors to condemn the clinical use of this x-ray contrast agent in human patients.—C. A.

Recent Trends in Radiation Therapy of Cancer. PETTIT, R. T. [Ottawa, Canada] *Illinois M. J.,* **87**:232-236. 1945.

A discussion. The discovery that cells, normal and pathological, are much more susceptible to radiation effects during mitosis than in the resting stage has led to fractionating the radiation dosage and prolonging the period of time of administration over a number of days in order to affect many more cells during mitosis. The author feels this method has more to offer than the massive dose technic, even in carcinoma of the lip. The proper selection of wave length (voltage and filtration)

is also important. The author decries the more recent trend toward the use of multiple small fields and favors, for many reasons cited, a larger field of radiation therapy. He calls attention to the fact that radiation sensitivity and curability of the lesion are by no means synonymous. He advocates the present day trend in the better clinics to consider a patient as having some chance for a cure in almost every instance and to treat adequately rather than palliatively. To be effective, radiation therapy must be carried out intensively at the first attempt. In many instances the combined use of radium and x-rays is essential. In the author's experience during the past 2 years, x-rays from a 400,000 volt machine have given better results in carcinoma of the cervix, rectum, and bladder and in osteogenic sarcoma than were previously obtained with ordinary "deep x-ray therapy" at 200,000 volts.—M. E. H.

Comparison of Physical and Biological Methods of Depth Dose Measurement. Packard, C., and Exner, F. M. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Radiology,* **44**:357-366. 1945.

Values for the ratio of depth to surface dose obtained by a physical method (Victoreen dosimeter) agreed, within the limits of experimental error, with those obtained by a biological method (Drosophila eggs).—R. E.S.

Measurements of Surface and Depth Dose Ratios from 70 to 1,000 Kv. Exner, F. M., and Packard, C. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Radiology,* **44**:367-400. 1945.

Curves for the measurement of surface and depth dose ratios at voltages of from 70 to 1,000 kv. are given. These charts with instructions for their use are obtainable at cost.—R. E.S.

X-Ray Treatment of Skin Cancer. Derr, J. S. [Frederick, Md.] *Urol. & Cutan. Rev.,* **49**:287-290. 1945.

The author reviews 409 cases of skin cancer treated by himself with x-radiation and concludes that this is the method of choice. Late sequelae and the exact number of recurrences are not reported.—V. F. M.

Cancer cutané stérilisé par le radium après des délais anormaux. [Skin Cancer Cured by Radium Therapy after an Unusual Lapse of Time.] Wallon, W. [Anticancer Center, Salpêtrière, Paris, France] *Presse méd.,* **53**:106-107. 1945.

A case report. An epidermoid carcinoma of the labia majora was treated by radium. A biopsy taken 2 months later revealed the persistence of neoplastic cells. The tumor continued to regress without further applications. During a follow-up of 6 years, there has been no recurrence.—C. A.

Le traitement du Cancer des paupières. [The Treatment of Cancer of the Eyelids.] Laborde, S., and Billiard, J. [Cancer Inst., Univ. of Paris, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **29**:59-69. 1940.

The cure of cutaneous cancer of the eyelid and neighboring tissues presents an important therapeutic problem. Treatment by means of radium needles produced cures with a minimum of residual deformity in 65 of 82 cases and avoided irradiation-lesions of the neighboring tissues.—G. H. H.

Radiation of the Ear. Perlman, H. B. [Univ. of Chicago, Chicago, Ill.] *Laryngoscope,* **54**:255-266. 1944.

The effect of radiation of the normal ear, the injured ear, and the ear with obstruction of the eustachian tube by carcinoma of the nasopharynx was studied. In the last group of cases there was initial conduction deafness, which was completely reversed by massive radiation with x-rays, in doses up to 15 r per minute and 300 r per day, totalling about 5,000 r.—W. A. B.

Sur le diagnostic radiologique des cancers du larynx. [The Radiologic Diagnosis of Cancer of the Larynx.] Mathey-Cornat, R. [Pellegrin-LeTondu Hosps., and Sch. of Med., Bordeaux, France] *Presse méd.,* **53**:395-396. 1945.

A discussion on the x-ray diagnosis of cancer of the larynx with statistical report on 220 cases.—C. A.

Radiosensibilité et métastases observées au cours des cylindromes et des tumeurs mixtes des glandes salivaires. [Radiosensitivity and Metastases Observed in Cylindromas and Mixed Tumors of the Salivary Glands.] Baclesse, F. [Curie Foundation, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer,* **29**:260-274. 1940-1941.

A study of 18 cases of salivary gland tumors (9 mixed tumors of the parotid, 5 cylindromas of the submaxillary gland, 4 cylindromas of the buccorhinopharyngeal region) treated by radiotherapy. Recurrences were common after local excision. One visceral metastasis from the mixed tumors and 7 from the cylindromas were observed over a period of 3 to 22 years after diagnosis. Neither type of tumor responded to radiotherapy.—G. H. H.

Diagnostic précoce d'un petit ulcère gastrique en voie de cancérisation, selon la méthode radio-clinique de R. A. Gutmann. [Early Diagnosis of a Small Gastric Ulcer Undergoing Cancerous Transformation, by the Radiological-Clinical Method of R. A. Gutmann.] Albot, G., Parturier-Lannegrace, M., and Perrot, M. [Cancer Inst., Paris, France] *Presse méd.,* **49**: 1346-1348. 1941.

A case report. The early diagnosis was made on the persistence of a rigid "niche" after the clinical signs had regressed.—C. A.

Bone Tumours and Their Radiological Implications. Grout, J. L. A. *Proc. Roy. Soc. Med.,* **38**:345-347. 1945.

The speaker calls attention to the difficulties in radiological interpretation and the misunderstandings liable to occur if too much stress is laid on radiographic features without reference to the clinical findings. Points are illustrated by a series of radiographs of bone tumors collected over a period of 20 years.—W. V. M.

Skin and Subcutaneous Tissues

Problèmes cliniques posés par les tumeurs noirs de la peau. [The Clinical Problems of Pigmented Tumors of the Skin.] Roussy, G., Huguenin, R., and Saracino, R. [Cancer Inst., Paris, France] *Presse méd.,* **50**: 193-197. 1942.

A discussion of the clinical signs and treatment of pigmented moles, nevocarcinoma, and secondary pigmented

carcinoma of the skin, with short case reports and photographs.—C. A.

Angiokeratoma. Sherry-Dottridge, F. *Proc. Roy. Soc. Med.*, **38**:334. 1945.

Description of a case.—E. L. K.

Case of Multiple Minute Fibro-Angiomata of Face and Ears (Pringle's Disease, Darier's type). Roxburgh, A. C. *Proc. Roy. Soc. Med.*, **38**:331-332. 1945.

Description of a case.—E. L. K.

Breast

Low-Back Pain as the Presenting Symptom of Malignant Breast Tumors. Cohn, T. D., and Cohn, H. *New England J. Med.*, **232**:342-343. 1945.

Four cases of carcinoma of the breast are reported in which pain from metastatic foci to the spine and bony pelvis was the first evidence of neoplastic disease.—C. W.

Clinical Effects of Surgical and X-Ray Castration in Mammary Cancer. Adair, F. E., Treves, N., Farrow, J. H., and Scharnagel, I. M. [Memorial Hosp., New York, N. Y.] *J. A. M. A.*, **128**:161-167. 1945.

This study is a review of all patients with carcinoma of the breast who have been castrated with surgery or radiation in a period of 18 years at the Memorial Hospital. Improvement was noted in approximately 13 to 15% of 335 women so treated. There seemed to be no difference between x-ray castration and surgical castration. It would appear that, among women, castration gives a temporary, rather than a permanent, improvement.

More striking effects from castration were noted among 7 males with breast carcinoma. The change of the disease processes seems to be immediate, and the improvement in general health is as striking as that seen in cases of carcinoma of the prostate treated by castration.—M. E. H.

Primary Lymphosarcoma of the Breast. Adair, F. E., and Herrmann, J. B. [Memorial Hosp., New York, N. Y.] *Surgery*, **16**:836-852. 1944.

In primary lymphosarcoma of the breast, there may be a single focus, or multiple foci as in 1 of the 5 cases reported. Three of these patients had radical mastectomies, 1 a simple mastectomy, and the fifth, local excision followed by irradiation. All 5 patients are alive from 3 months to 7 years after therapy. Radical operation is the treatment of choice except in cases in which the disease is not limited to the breast; the latter are best treated by irradiation alone.—W. A. B.

Female Genital Tract

Androgenic Therapy in Malignant Disease of the Female Genitalia. Abel, S. [Passavant Hosp., and Northwest. Univ. Med. Sch., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **49**:327-340. 1945.

Testosterone propionate therapy (140 to 150 mgm. weekly) was utilized in the treatment of patients with progressively advancing carcinoma of the uterine corpus, cervix, fallopian tube, ovary, or breast. Symptomatically the patients were improved. There was no indication, however, of any regression or even retardation of the malignant process. The dose of androgen used was sufficient to cause masculinization.—A. K.

Three Cases of Solid Tumours of Ovary. I. Papillary Cystic Carcinoma. II. Granulosa-Cell Carcinoma. III. Papillomatous Adenoma. Kelson Ford, R. *Proc. Roy. Soc. Med.*, **38**:330. 1945.

Descriptions of cases.—E. L. K.

Theca Cell Tumor of the Ovary in a 72 Year Old Woman. Wimpfheimer, S. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:768-772. 1945.

Report of a case of theca-cell tumor of the ovary, which ocurred in a 72 year old woman, 21 years after the menopause. The feminizing effect produced uterine bleeding and enlargement of the breasts.—A. Cnl.

Dermoid Cyst of Ovary Containing a Structure Similar to a Human Body (Homunculus). Plaut, A. [Beth Israel Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**: 567-578. 1945.

A dermoid cyst is described containing, free in the lumen, a miniature human-like structure (homunculus) with remnants of an umbilical cord. Pertaining literature and theories are discussed with emphasis on the organizer theory.—Author's summary. (A. Cnl.)

The Value of Vaginal Smears in the Diagnosis of Early Malignancy. Jones, C. A., Neustaedter, T., and Mackenzie, L. L. [New York Post-Grad. Med. Sch. and Hosp., Memorial Hosp., and New York Infirmary, New York, N. Y.] *Am. J. Obst. & Gynec.*, **49**:159-168. 1945.

The vaginal smear method, used in 427 cases, led to a diagnosis of cancer of the cervix or fundus of the uterus in 91, and in 82 of these the diagnosis was confirmed by biopsy or curettage. There were 7 false negative diagnoses. The method is recommended for continued trial as a preliminary or corroborative measure.—A. K.

Le cancer de l'utérus et sa prévention. [Cancer of the Uterus and Its Prevention.] Laborde, S. *Presse méd.*, **50**:739-740. 1942.

A study of the predisposing factors in uterine cancer and of ways of controlling this type of growth.—C. A.

Adenoacanthoma of Uterus. Ayre, J. E. [Roy. Victoria Hosp., McGill Univ., Montreal, Canada] *Am. J. Obst. & Gynec.*, **49**:261-264. 1945.

Case report. There was evidence of malignant change in both squamous and glandular tissue in the body of the uterus. The abnormally placed squamous epithelium is considered to have arisen by stromal metaplasia.—A. K.

Heterologous Mesodermal Tumors of the Uterus. Report of a Neoplasm Resembling a Granulosa Cell Tumor. Morehead, R. P., and Bowman, M. C. [Bowman Gray Sch. of Med. of Wake Forest Coll., Winston-Salem, N. C.] *Am. J. Path.*, **21**:53-61. 1945.

Case report with 7 photomicrographs.—J. G. K.

Résultats éloignés de la méthode combinée (radium et hystérectomie complémentaire) dans le traitement des cancers-cervico-utérins. [Results Obtained with the Combined Treatment (Radium and Secondary Hysterectomy) of Cancer of the Cervix.] Brénier, J. L. [Paris, France] *Presse méd.*, **53**: 338-339. 1945.

Thirty-nine of 51 patients are reported cured by this

method after periods varying from 6 to 19 years. The surgical mortality was 4%.—C. A.

Early Cervical Carcinoma in Three Clinically Unsuspected Cases Incidental to Plastic Operations. Rubin, I. C. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:607-615. 1945.

Three cases of early cancer of the cervix are reported. One tumor was discovered in small cervical fragments removed during a trachelorrhaphy. This patient was followed for 7 years; there was no evidence of recurrence or further growth. In the 2 remaining cases, the tumor was discovered in the amputated cervix after Fothergill operations; 1 patient developed recurrence in the cervical stump 5 years and 4 months after the operation, despite x-ray and radium therapy, and the other, also treated, at 5 years after operation had shown no recurrence. The question of latency and the morphologic criteria of early cancer are discussed.—A. Cnl.

Carcinoma of the Cervix and Pregnancy: A Clinical Study of Eight Cases. Kobak, A. J., Fitzgerald, J. E., Freda, V. C., and Rudolph, L. [Cook Co. Hosp., Illinois Univ., Northwest. Univ., and Loyola Univ., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **49**:307-321. 1945.

In 7 of the patients described, the tumor was a stratified squamous cell carcinoma, and in 1 it was an adenocarcinoma.

One patient aborted after radium treatment, 4 were delivered by cesarean section, 1 gave birth spontaneously, 1 was subjected to hysterectomy after death of the fetus, and 1 died undelivered. Bleeding during pregnancy was reported by 6 of the 8 patients; obstetricians are urged to suspect carcinoma in the presence of this symptom. Treatment for various stages is indicated.—A. K.

Papillary Lesions of the Cervix Uteri in Pregnancy. Edmondson, H. A., Levi, L. M., Evans, N., and Horn, P. [Los Angeles Co. Hosp., Univ. of South. California, and Coll. of Med. Evangelists, Los Angeles, Calif.] *Am. J. Obst. & Gynec.*, **49**:356-367. 1945.

Five instances of proliferative or papillary lesions of the cervix uteri associated with pregnancy are reported. The ages of the patients ranged from 17 to 22 years. The lesions were seen at various stages of pregnancy, from the second month to time of delivery. The proliferative growths were single or multiple, varied in size from small pearly gray-white areas to papillomas raised 2 to 3 cm. above the surface and were always located at or near the external cervical os. There were various grades of squamous epithelial proliferation microscopically, *i.e.*, (a) simple thickening, (b) greatly thickened irregular epithelial units fused together with confluent tiny papillary stalks, (c) large papillomatous growths. Irregularity in size of nuclei, hyperchomatism, mitotic activity, and cytoplasmic vacuolization tend to confuse the diagnosis with carcinoma, but true invasion does not occur. The etiology is not clear. Large discrete tumors can be removed surgically. Radiation is of value in treating extensive multiple lesions.—A. K.

Squamous Metaplasia of the Cervix Uteri. Auerbach, S. H., and Pund, E. R. [Univ. of Georgia Sch. of Med., Augusta, Ga.] *Am. J. Obst. & Gynec.*, **49**:207-213. 1945.

This report is based on sections of 600 cervices. The authors believe that chronic inflammation is an important factor in inducing replacement of columnar epithelium by stratified squamous epithelium within the cervical canal. Another factor that may be of major importance is hyperestrinism. It is thought that the metaplastic epithelium probably does not give rise to cancer of the cervix, but that both the cancerous and metaplastic epithelium have a common ancestral cell.—A. K.

Sarcoma of Cervix; Primary Carcinoma of Fallopian Tube; Primary Sarcoma of Fallopian Tube. Rickford, B. *Proc. Roy. Soc. Med.*, **38**:321-323. 1945.

Descriptions of cases.—E. L. K.

Urinary System—Male and Female

Wilms' Tumor of Kidney With Five Year Survival Following Preoperative Roentgen Therapy and Nephrectomy. Mandeville, F. B., and Nelson, C. M. [Med. Coll. of Virginia Hosp., Richmond, Va.] *Urol. & Cutan. Rev.*, **49**:204-208. 1945.

A case report with discussion and roentgenograms.—V. F. M.

Wilms' Tumor of the Kidney. Giles, R. G. [San Antonio, Tex.] *Urol. & Cutan. Rev.*, **49**:217-220. 1945.

This is a presentation of 4 fatal cases and a discussion of the subject. Irradiation before and after nephrectomy is advised.—V. F. M.

Conservative Surgery in Certain Benign Tumors of the Ureter. Vest, S. A. [Univ. of Virginia Hosp., Charlottesville, Va.] *J. Urol.*, **53**:97-121. 1945.

The author advocates local removal of well pedunculated ureteral tumors. Three case reports are presented, with illustrations, and the literature is extensively reviewed.—V. F. M.

Malignant Granular Cell Myoblastoma Involving the Urinary Bladder. Ravich, A., Stout, A. P., and Ravich, R. A. [Adelphi Hosp., Brooklyn, N. Y., and Columbia Univ., New York, N. Y.] *Ann. Surg.*, **121**:361-372. 1945.

A case is reported in which death occurred from a metastasizing tumor diagnosed as granular cell myoblastoma, which has formerly been considered benign.—W. J. B.

Épithélioma primitif de l'urètre penien. [Primary Epithelioma of the Penile Urethra.] Macquet, P., and Verhaeghe, M. [Sch. of Med., Lille, France] *Presse méd.*, **51**: 512-513. 1943.

A case report.—C. A.

Salivary Glands

Le problème de la malignité des tumeurs mixtes des glandes salivaires. [The Problem of the Malignancy of Salivary Gland Mixed Tumors.] Gricouroff, G. [Radium Inst. of Paris, and Curie Foundation, Paris, France] *Presse méd.*, **52**:646-647. 1943.

A discussion. Mixed tumors of salivary glands are not carcinomas, but should not be treated as benign tumors.—C. A.

A propos du traitement des tumeurs dites "mixtes" de la parotide. [The Treatment of "Mixed" Tumors of the Parotid.] REDON, H. [Cancer Inst., Paris, France] *Presse méd.*, **51**:242-243. 1943.

A discussion. Total parotidectomy with conservation of the facial nerve is the treatment of choice.—C. A.

Tumeurs mixtes de la parotide. [Mixed Tumors of the Parotid.] ROUX-BERGER, J.-L. [Tenon Hosp., and Curie Foundation, Paris, France] *Presse méd.*, **48**:971-973. 1940.

Report of 7 cases. Complete surgical enucleation of such tumors being extremely difficult, total parotidectomy is the only logical treatment.—C. A.

Primary Tumors of Stensen's and Wharton's Ducts. FIGI, F. A., and ROLLAND, W. D. [Mayo Clin., Rochester, Minn.] *Arch. Otolaryng.*, **40**:175-176. 1944.

A report of 4 cases in 2 of which the lesion proved to be a primary adenocarcinoma. In the others symptoms were due to an inflammatory nodule; a diagnosis of salivary calculus had been excluded preoperatively by x-ray examination.—W. A. B.

INTRATHORACIC TUMORS—LUNGS—PLEURA

L'étude des cancers professionnels est-elle susceptible d'apporter de nouvelles notions étiologiques et pathogéniques en ce qui concerne le cancer des bronches? [Can the Study of Occupational Cancers Further Our Knowledge of the Etiology and Pathogenesis of Bronchial Carcinoma?] LECOEUR, J. *Presse méd.*, **50**:415-417. 1942.

A better clinical study of occupational bronchial carcinoma caused by asbestos, chrome, and radium is advocated. Effort should be made to produce these tumors in experimental animals.—C. A.

Disease of Lungs in Boiler Scalers, with a Case Report and Review of the Literature. HARDING, H. E., TOD, D. L. MCR., and MCLAUGHLIN, A. I. G. [Univ. of Sheffield, Anlaby Road Hosp., Hull, and Factory Dept., Ministry of Labour and Nat. Service, England] *Brit. J. Indust. Med.*, **1**:247-251. 1944.

A man who had been engaged as a marine boiler scaler for 47 years died of metastatic bronchogenic carcinoma possibly initiated by a carcinogenic agent in the flue dust or soot. His lungs showed silicosis, and pigmentation due to iron. A sample of scale taken from the type of boiler usually scaled by this man contained 10.1% Fe, 8.6% total silica; a sample of flue dust contained 48.3% Fe, 6.1% total silica, and 27% combustible material (soot).—M. H. P.

A propos du diagnostic précoce du cancer pulmonaire primitif. [The Early Diagnosis of Primary Cancer of the Lung.] LAUMONIER, P., and LELOURD. [Xavier-Arnozan Sanatorium, and St. André Hosp., Bordeaux, France] *Presse méd.*, **49**:654-656. 1941.

A review with case reports.—C. A.

Tumeurs végétantes endobronchiques guéris par pneumectomie totale. [Endobronchial Vegetative Tumors Cured by Total Pneumectomy.] SANTY, M. P., and BÉRARD, M. *Presse méd.*, **50**:709-710. 1942.

Two case reports of bronchial adenoma treated by total pneumectomy. The patients are well 3 years and 1 year, respectively, after operation.—C. A.

Sur une forme particulière d'épithéliomas des grosses bronches. Les "épistomes" bronchiques. [A Special Type of Epithelioma of the Large Bronchi. Bronchial "Epistomas."] PRUVOST, P., JACOB, P., DELARUE, and DEPIERRE. [Paris, France] *Presse méd.*, **49**:1140-1143. 1941.

A report of 2 cases of mixed tumors of the lung, so-called bronchial adenomas.—C. A.

Nécessité de la biopsie pleurale pour le diagnostic de l'endothéliome de la plèvre. [The Necessity of a Pleural Biopsy in Establishing a Diagnosis of Pleural Endothelioma.] FOURESTIER, M., and DURET, M. [Paris, France] *Presse méd.*, **51**:467-468. 1943.

Three case reports demonstrating how pleuroscopy together with pleural biopsy is the only way of making a differential diagnosis between endothelioma and subpleural cancer of the lung.—C. A.

GASTROINTESTINAL TRACT

Transthoracic Esophagogastrostomy for Carcinoma of the Middle Third of the Esophagus. Report of a Successful Resection. CLARK, D. E. [Univ. of Chicago, Chicago, Ill.] *Ann. Surg.*, **121**:65-73. 1945.

A case report.—W. J. B.

La position médico-chirurgicale actuelle du cancer de l'estomac en pratique. [The Medico-Surgical Aspect of Gastric Cancer Today.] PAPIN, F. [Sch. of Med., Bordeaux, France] *Presse méd.*, **49**:545-548. 1941.

A discussion with special reference to early diagnosis and surgical technic in gastric cancer.—C. A.

Diagnostic précoce d'un cancer fibreux "incipiens" de la petite courbure gastrique. [Early Diagnosis of an "Incipient" Fibrous Carcinoma of the Lesser Curvature of the Stomach.] CHIRAY, M., ALBOT, G., DEBRAY, C., and LECANUET, L. [Paris, France] *Presse méd.*, **50**:197-199. 1942.

A case report. Early diagnosis of a small gastric carcinoma of the linitis type.—C. A.

Cancer primitif de l'estomac "in situ," strictement localisé à la muqueuse. Dépistage précoce radioclinique et gastroscopique. [Gastric Carcinoma "in Situ," Strictly Limited to the Mucosa. Early Diagnosis by X-Ray, Gastroscopic and Clinical Signs.] ALBOT, G., GOSSET, J., BERTRAND, I., and PARTURIER-LANNEGRACE, M. [Cancer Institute, Univ. of Paris, and Salpêtrière, Paris, France] *Presse méd.*, **50**:711-712. 1942.

A case report.—C. A.

Diagnostic gastroscopique d'un cancer ulcérant de l'estomac au début. Contrôle radio-endoscopique de son évolution. [Gastroscopic Diagnosis of an Early Ulcerating Gastric Carcinoma. X-Ray and Endoscopic Study of Its Course.] MOUTIER, F. [Broussais Hosp., Paris, France] *Presse méd.*, **51**:591-592. 1943.

A case report.—C. A.

Diagnostic précoce du cancer gastrique. [Early Diagnosis of Gastric Cancer.] GUTMANN, R. A. [Salpêtrière, Cancer Inst., and Hôtel-Dieu, Paris, France] *Presse méd.*, **53**:150-151. 1945.

A report on the follow-up of 18 cases of carcinoma

"in situ" and small cancerous lesions of the stomach, in which an early diagnosis was followed by prompt surgical treatment. Only 1 death is recorded; it occurred after hepatic metastases.—C. A.

Cancer primitif de l'estomac "in situ." [Primary Gastric Cancer "in Situ."] ALBOT, G., LÉGER, L., and YCRE, R. [Salpêtrière, Paris, France] *Presse méd.*, **51**:313-314. 1943.

A case report.—C. A.

Partial Gastrectomy for Carcinoma Thirty-Five Years Ago. LUND, F. B. [Boston City Hosp., Boston, and Quincy City Hosp., Quincy, Mass.] *New England J. Med.*, **232**: 562-564. 1945.

A case of a 35 year survival following a Billroth No. 2 operation for a poorly differentiated, rapidly growing carcinoma is presented with a photomicrograph of the original section.—C. W.

L'apparition du cancer gastrique chez les sujets atteints d'anémie de Biermer. [The Occurrence of Gastric Cancer in Patients with Pernicious Anemia.] FIESSINGER, N., ALBEAUX-FERNET, M., and LAJOUANINE, P. *Presse méd.*, **49**:809-812. 1941.

Report of 2 cases.—C. A.

Schwannome de l'estomac. [Gastric Schwannoma.] PÉRIER, E. A. [Salpêtrière, Paris, France] *Presse méd.*, **53**:134-135. 1945.

Three case reports.—C. A.

Postoperative Survival Periods in Gastro-Intestinal Carcinomata. APPLEBY, L. H. [Vancouver, Brit. Columbia, Canada] *Canad. M. A. J.*, **52**:481-484. 1945.

This is a statistical study of the end results of surgical treatment for carcinoma of the gastrointestinal tract in more than a thousand cases during a 20 year period. Surgery for gastric carcinoma in 193 cases provided some relief from pain and distress but, through the years, no cures. In carcinoma of the intestines, 287 resections were followed by 8% mortality and 31% 5 year cures.—M. E. H.

Primary Carcinoma of Third Portion of the Duodenum. SHALLOW, T. A., EGER, S. A., and CARTY, J. B. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Surgery*, **16**:939-946. 1944.

A carcinoma of the third portion of the duodenum was resected, and an antecolic duodenojejunostomy performed. The patient, a 63 year old male, was apparently well 9 months after the procedure.—W. A. B.

Malignant Carcinoid (Argentaffinoma) of the Ileum with Widespread Metastases. TUTA, J. A. [Grant Hosp. of Chicago, and Univ. of Illinois Sch. of Med., Chicago, Ill.] *Illinois M. J.*, **87**:200-202. 1945.

A case of a malignant carcinoid of the lower ileum with widespread metastases to the adjacent mesentery, to the periaortic, peripancreatic, and posterior mediastinal lymph nodes, and to the liver, pancreas, and heart is reported. Among 50 malignant carcinoids of the small intestine reported in the literature, only a few have shown widespread metastases.—M. E. H.

Sur la résection en un temps des cancers du colon transverse, et sur l'iléostomie complémentaire dans les interventions sur le gros intestin. [The Resection in One Stage of Cancers of the Transverse Colon, and Complementary Ileostomy in Surgery of the Large Intestine.] LENORMANT, C. [Paris, France] *Presse méd.*, **49**:281. 1941.

Two case reports.—C. A.

Bowel Surgery. BERGER, L., and HIRSCH, E. [Jewish Hosp. of Brooklyn, Brooklyn, N. Y.] *Am. J. Surg.*, **66**:31-43. 1944.

The series of surgical cases reviewed comprises 116 cases of cancer of the large bowel and 18 cases of other lesions. The role of preoperative care in increasing extent of operability is stressed. The surgical procedures chosen were: anastomosis and immediate resection in right and mid-colon growths, obstructive resections with delayed anastomosis in left colon growths, and one stage combined abdominoperineal resections for rectal carcinoma.—W. A. B.

Complications in the Surgical Treatment of Carcinoma of the Large Bowel. BABCOCK, W. W., and BACON, H. E. [Temple Univ. Sch. of Med., Philadelphia, Pa.] *J. A. M. A.*, **128**:73-77. 1945.

On the basis of 712 operations for cancer of the colon, the authors discuss the choice of operation, wound complications, drainage, the preservation of a functional anal orifice, as well as postoperative pulmonary complications. They have adopted radical excision instead of colostomy for patients with advanced secondary lesions from cancer of the large bowel and have tended toward a wider use of end to end resection, preservation of the uninvolved anus, and the elimination of the Mikulicz operation except when serious peritoneal contamination renders a rapid termination of the intra-abdominal procedure desirable. Statistics are presented in support of these decisions.—M. E. H.

MISCELLANEOUS

Sur la récidive du cancer dans la cicatrice opératoire. [The Recurrence of Cancer in the Surgical Scar.] GRICOUROFF, G. *Presse méd.*, **50**:388-390. 1942.

A discussion on the pathogenesis of cancerous nodules in surgical scars. The author favors the theory of retrograde metastasis.—C. A.

Book Reviews

ADVANCES IN ENZYMOLOGY AND RELATED SUBJECTS OF BIOCHEMISTRY. Volume V. Edited by F. F. Nord and C. H. Werkman. New York: Interscience Publishers, Inc., 1945, vii + 268 pages. Price $5.50.

This is the fifth volume in the series of reviews by authorities on various aspects of enzymology. The editors and publishers are to be congratulated on maintaining the high standard set by the earlier volumes, and on sustaining and apparently encouraging the free expression of opinion by their contributors. These reviews are in no sense a series of annotated bibliographies, but rather are scholarly and critical expositions of various fields of enzymological research, frequently seasoned by the wit and by the personal philosophical disquisitions of the authors.

In the volume under review, it is interesting to note that several of the authors, Nord and Leibowitz in particular, have commented on the perennial question of just how far studies on isolated catalytic systems may be applied to the living cell. The last section in the chapter by Leibowitz is especially recommended as a provocative presentation of the issues involved and is amusingly illustrated by the case histories of Willstätter and of Warburg, whose original postulates, conceived in opposite directions, were negated by their own subsequent work. A teleological enzymologist might quarrel with the conclusion that "the enzymatic processes of cell extracts . . . . disclose potential mechanisms of, but not necessarily mechanisms operative in, the living organism" (p. 124). Perhaps there would be more general agreement with this statement if it were amended by the insertion of the phrase "at maximum capacity" after the word "operative." The living cell follows a metabolic pattern inherent in the systems that it possesses and in the substrates available to it. It may well be that the organized cell does not employ at any one instant all the systems revealed in the cell extract or, of these, any one at its maximum capacity; but it is difficult to conceive after so many aeons of evolutionary development that there should be any useless cellular components. The reviewer is content to view the phenomena of the cell extract as a reflection, imperfect perhaps, of the metabolic functions of the living cell, and to let it go at that.

Despite the fact that no easy answer is available, it is good to examine thoughtfully every so often the bases upon which any experimental approach rests. The enzymologist may employ as the source of catalytic systems individual components of cells, cell extracts, broken cell suspensions, surviving individual cells, tissue slices, whole organs, or the living animal. Each approach has its creative value, and none is inherently superior to the rest. As long as the limitations imposed by each technic are appreciated, its employment in adequate experiment may follow, and the knowledge obtained thereby integrated with that derived from other approaches. A determinedly optimistic approach, even with seemingly inadequate tools, often works wonders.

The first article in the volume is on the Physical and Chemical Properties of Tomato Bushy Stunt Virus and the Strains of Tobacco Mosaic Virus, by N. W. Pirie, of the Rothamsted Experimental Station, in England. As might be expected from this excellent worker in the virus field, the article presents a critical and incisive survey of the purified plant viruses. Much of our knowledge of the chemical and physical properties of the viruses has been obtained from studies of the relatively abundant plant materials, although it is understood that certain of the animal viruses may differ from these in many particulars. Pirie points out that the study of the purified viruses may be used as illuminating exercises in protein chemistry, for they possess many unique characteristics susceptible to quantitative analysis. The repeated use by the author of the word "guanidine," when he means guanidine hydrochloride, is a little misleading, as implying that all guanidine salts are equally effective; guanidine sulfate, for example, is not the denaturing agent that the halides are. The review closes by dismissing the question of the living nature of viruses as a problem in semantics.

E. Chargaff has written an excellent review of the Coagulation of Blood, from a limited but well-seasoned point of view. Chargaff's review lays emphasis on the individual components of the blood coagulation mechanism, in which field he has made notable contributions, and, as a whole, is a lucid contribution to the understanding of this complicated problem.

The third review is an article by H. Blaschko on Amino Acid Decarboxylases of Mammalian Tissue. These are enzymes which, by catalyzing the decarboxylation of amino acids, leads to the formation of pharmacologically-important amines such as histamine, tyramine, and so forth. The review is valuable and excellently constructed.

The review by H. Leibowitz and S. Hestrin on the Alcoholic Fermentation of the Oligosaccharides is of particular contemporary interest, not so much because of the finely presented subject matter, but because of the viewpoint offered. The problem raised by these authors is, essentially, whether the oligosaccharides, maltose, lactose, fructose, etc., are fermented as such or whether they are first hydrolyzed and the resulting monosaccharides then fermented. The development of the problem is brilliantly presented and its sequelae are lucidly discussed. The authors incline to the interpretation that fermentation takes place at the disaccharide stage, but they reveal frankly the difficulties in the way of reproducing such an event in isolated, noncellular enzyme systems. The problem of whether metabolism takes place with large molecules or only after their hydrolysis into smaller fragments, is a general one. It will be recalled that desamination of the purine base in various nucleotides can occur without previous hydrolysis of the base, and indeed the isolation by Liebig of inosinic acid (the desaminated form of adenylic acid) antedated the discovery of the parent

nucleotide. The late Max Bergmann believed that amino acids are metabolized while they are yet in peptide linkage, and following this lead others observed the desulfhydration of cystine in peptide combination. The desamination of nucleic acids in the form of nondialyzable, polymerized tetranucleotides is yet another example. It appears that various kinds of substrates can be metabolized while they are in combination in the form of large molecules and before hydrolysis to the free substances occurs. A picture of piecemeal metabolism is thus drawn.

The review by E. Stotz on Pyruvate Metabolism is an excellent and clear collection of the facts and theories relating to this complicated problem. As the author points out, a sharper focus on pyruvate has quite properly displaced the emphasis formerly placed on lactate, since the latter arises only secondarily by reduction by pyruvate and its immediate fate is to be reconverted to pyruvate. The relation of diphosphothiamine (phosphorylated vitamin B_1) to pyruvate metabolism is well presented, as is the connection of pyruvate with certain clinical problems. In discussing amino acid sources of pyruvate, the author neglected to mention the fact that cystine in the presence of liver readily yields pyruvate, indeed more readily than alanine.

Recent Progress in the Biochemistry of *Fusaria* is the title of an article by F. F. Nord and R. P. Mull. The metabolism of this organism is described at some length. An interesting feature of this metabolism is the ability of elemental sulfur to serve as a hydrogen acceptor in the course of carbohydrate fermentation. The large amount of data available on this organism is astonishing, but their importance becomes apparent in the last few sections of the review when technological applications are considered. The bulk of the sugars formed in the process of paper pulp manufacture is composed of pentoses that are fermentable only by *Fusaria*. The high vitamin B_1 content of this organism opens up another source of application.

The review by F. Schlenk on Enzymatic Reactions Involving Nicotinamide and its Related Compounds is a valuable article on the role that the pyridine nucleotides play in various enzymatic processes. These nucleotides are the coenzymes of dehydrogenase systems and participate in reversible oxidation-reduction equilibria. A section is devoted to Kensler's fine experiments on the inhibition of yeast fermentation by various products found in the urine after the feeding of *p*-dimethylaminoazobenzene. It should be stated that the substances that Kensler found to be inhibitory were not carcinogenic, while those that were carcinogenic were not inhibitory. Further study of codehydrogenases in tumors is desirable.

The last review in the volume, by C. V. Smythe, on Some Enzyme Reactions on Sulfur Compounds, is a brief but highly satisfactory article on the transformations that the sulfur-containing amino acids undergo. Smythe has made notable contributions to this subject, particularly in the enzymatic desulfhydration of cystine and cysteine to hydrogen sulfide, ammonia, and pyruvic acid; by ingenious experiments involving radioactive sulfur he proved the reversibility of this reaction. The reviewer enjoyed the article, not only because of his high regard for Smythe, but because it reminded him of a moral-pointing episode. Several years ago the reviewer was studying the action of various tissues in splitting cystine peptides, and noted that with liver these substrates, instead of yielding a clean precipitate of free cystine, gave an evil mess smelling strongly of hydrogen sulfide. With a minimum of thought, the material was poured down the sink and forgotten. The following year, Fromageot and his associates announced the presence in liver of an enzyme that split hydrogen sulfide from cystine.

JESSE P. GREENSTEIN

A SYMPOSIUM ON MAMMARY TUMORS IN MICE. Publication of the American Association for the Advancement of Science No. 22. By Members of the Staff of the National Cancer Institute, Bethesda, Md. Edited by F. R. Moulton. Lancaster: Science Press, 1945. 223 pages. Price $4.00.

Ten investigators in the National Cancer Institute have contributed twelve articles covering every aspect of our knowledge of mammary cancer in the laboratory mouse. The table of contents gives a fair idea of the scope of the work:

General and Historical Introduction . . . . . M. B. SHIMKIN
Cytology of Mammary Tumors of the Mouse . . . . . A. J. DALTON
Morphology and Histogenesis of Mammary Tumors . T. B. DUNN
Histogenesis of the Mammary Gland of the Mouse . A. J. DALTON
The Vascular Supply of Mammary Gland Carcinomas
G. H. ALGIRE and H. W. CHALKLEY
Genetics of Mammary Tumors in Mice . . . . . W. E. HESTON
Hormones and Mammary Cancer in Mice . . . . . M. B. SHIMKIN
The Milk Influence in the Genesis of Mammary Tumors
H. B. ANDERVONT
Diet and Some Other Environmental Influences in the Genesis and Growth of Mammary Tumors in Mice
H. P. MORRIS
The Chemistry of the Mammary Tumors . . . . . J. P. GREENSTEIN
Experimental Treatment of Mammary Tumors in Mice
H. M. DYER
Conclusions—Including Discussion of the Possible Implications for Man . . . . . M. B. SHIMKIN

In this symposium one finds the latest and most complete knowledge of one of the most important and fruitful fields of cancer research. Obviously in such a compilation by several authors all aspects will not prove equally interesting to all readers or all articles seem equally complete.

To this reviewer the section by Andervont on the milk influence appears to be a very thorough and up-to-the-minute account of this most interesting and thought-provoking carcinogen. Included in the chapter are some previously unpublished observations that an antiserum to the milk factor can be made by injecting rabbits with a saline extract of mouse mammary tumor, an observation with tremendous therapeutic possibilities, if other cancers ever are shown to be dependent on similar factors.

The chapters by Shimkin, especially the concluding one of the book, are well written and packed with suggestions for further investigation. Shimkin seems to have no reticence in pointing out where our present knowledge is deficient and along what lines important discoveries may be made. His suggestion that the biochemist turn his attention to changes leading up to cancer rather than spending all his time on the frankly malignant and often degenerating tumor is a timely one. Also, the

proposal that Linderstrom-Lang's technics of histochemistry be made use of has interesting possibilities. This investigator has invented methods whereby enzyme concentrations can be studied in limited amounts of homogeneous tissues or even in cells themselves.

The book is an important one, certainly the most thorough, complete, and authoritative work dealing with mouse mammary cancer, and a "must" in the library of every laboratory worker in the field of cancer research.

GRAY H. TWOMBLY

AGEING AND DEGENERATIVE DISEASES. Edited by Robert A. Moore. Volume XI of BIOLOGICAL SYMPOSIA, a Series of Volumes Devoted to Current Symposia in the Field of Biology. Lancaster: Jaques Cattell Press. 1945 Preface and 242 pp. Price $3.00.

During a two day symposium sponsored by the Research Unit of the St. Louis City Infirmary and the Washington University School of Medicine in March, 1944, there were 13 papers presented. These now appear in book form. Six chapters in this volume are reviews of experimental work (4 related to arteriosclerosis, 1 to cancer, and 1 to nutrition). Three papers deal with clinical or postmortem studies (on glucose tolerance, on progeria, and on autonomic ganglia); the remaining 4 are discussions of degenerative diseases (recognition, treatment, research, and relationship to senescence).

Page gives a good review of the research and theories on atherosclerosis, but points out that progress on this most important problem has been discouragingly slow. Dragstedt discusses the experimental work on the pancreas and on lipocaic in relation to arteriosclerosis. Kountz and his collaborators bring out the fact that the incidence of atherosclerosis does not parallel the blood cholesterol levels. Hueper suggests that a number of materials or conditions may be involved in the etiology of atherosclerosis. Although frequent mention is made in these chapters of cholesterol and of cholesterol metabolism, there are indications of a growing feeling that the etiology of atherosclerosis involves other factors and other changes.

Leo Loeb gives an able discussion of research on cancer in relation to age. Saxton presents data on the longevity of rats in relation to their diets and to autopsy findings.

The difficulties in clinical recognition of degenerative diseases are discussed in a chapter by Stieglitz, who says that diagnosis of these diseases "is admittedly one of the most difficult and important problems in medicine." A chapter by MacNider deals with degenerative diseases in relation to senescence.

A case of progeria is presented by Schwartz and Cooke with a brief review of the cardiovascular changes observed in previous cases.

Kuntz gives information on the effects of degenerative diseases of the autonomic ganglia on the vascular system. He distinguishes between the changes that invariably occur with age and the pathological changes that occur irregularly.

Glucose tolerance in elderly patients is the subject of a chapter by Hofstatter and his collaborators, who state that "There is no evidence of impairment of the carbohydrate metabolism in ageing individuals to the degree that could be considered diabetic."

The weaknesses of the book (aside from the failure of the publishers to place the full title on the cover) lie, not in the presentations, but rather in the fact that we have been backward in our investigation of these highly important fields. There is a paucity of new information on the cardiovascular-renal diseases and on senescence, for which the authors are not at fault.

It is unfortunately true that gerontologists must constantly explain the purpose of (and even defend) their investigations on our most urgent medical problems. Saxton reiterates what has been said many times: "The aim in this study has not been to prolong the period of senility, but to investigate ways in which the productive life of the individual can be extended."

This volume presents a good cross section of our present knowledge on our most important medical problems.

HENRY S. SIMMS

KETTLE'S PATHOLOGY OF TUMOURS. By W. G. Barnard and A. H. T. Robb-Smith. Third Edition. London: H. K. Lewis & Co. Ltd. 1945, viii + 318 pages. Index included. 191 illustrations. Price: 21s.

It is a real pleasure to find that Kettle's book has reached a third edition, for it is by far the best short treatise on tumors within the knowledge of the reviewer.

The fear that it might follow its widely lamented author in death has now been lifted, and fortunate it is that the task of revision has fallen into such reverent hands. Much of Kettle's text has been preserved, together with the figures that he so painstakingly drew to illustrate it, and to this have been added all the most promising among recent discoveries. But so skillfully has this new material been woven in that it seems as though Kettle were still speaking to us of subjects that would have delighted him so: of the carcinogenic compounds, of the hormones, of the milk influence, and the like.

The hands are the hands of Esau, but the voice is Jacob's voice.

WILLIAM H. WOGLOM